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ABSTRACT

PLASTICITY OF THE CORTICAL REPRESENTATION OF FINGER EXTENSORS INDUCED BY PAIRED ASSOCIATIVE STIMULATION

by

Ian Anthony Gerard LaFond

This dissertation first explored associative plasticity of the human motor cortical representation with the use of noninvasive transcranial magnetic stimulation (TMS) paired with peripheral electrical stimulation. Paired Associative Stimulation (PAS) has grown in popularity because of its potential clinical applications. PAS techniques are used in combination with electromyography (EMG) measurements to study cortical excitability and features of hand movement. This work focuses on a cohesive approach to answer central questions about: the ideal mechanism to facilitate cortical plasticity via PAS, the interaction between the behavior performed and type of stimulation delivered to the targeted cortical network and the effects of PAS, the interaction between interstimulus timing, stimulus timing during movement and the translation of these effects into measurable changes starting from neurophysiological changes and ending up with the behavioral modulation of hand movement.

First the role of interstimulus timing and intracortical facilitation on modulation of cortical excitability is explored in the extrinsic hand muscles by showing that PAS can be conditioned by these facilitatory intracortical networks. Using standard indirect approaches utilizing peripheral EMG measures and novel virtual reality (VR) environments, a graded excitability response is shown for the PAS technique and illustrates that interactions of PAS with voluntary movements impacts the degree as well

as the state of cortical excitability. Rules governing the interactions of brain stimulation techniques and motor learning are important because brain stimulation techniques can be used to modify and improve neuro motor adaptation and skill learning with great potential for clinical applications such as facilitation of recovery after stroke. PAS provides us with a unique opportunity to study the rules of plasticity at a systems level, which is a combination of synaptic and non-synaptic (metaplastic) changes.

Finally, it is shown that changes in cortical excitability may help modulate certain neurophysiological and clinical features of hand function in a pair of patients with chronic stroke in a pilot study. As expected, stroke patients exhibited a smaller degree of excitability increase. It is demonstrated that sessions of intense training with PAS in a VR environment induces significant neuroplastic changes in the sensorimotor cortex. Explicitly, VR based PAS facilitates corticospinal excitability in the ipsilesional sensorimotor cortex. As a result, this dissertation provides a new methodological and technical framework to condition the standard PAS paradigm to engage other intracortical networks. It also shows how PAS can be used to affect motor learning and the role of state of cortical excitation in induction of homeostatic or non-homeostatic plasticity for patients with neurological and neuromuscular impairments for example stroke plus the potential behavioral consequences of PAS in human motor cortex to facilitate functional recovery of hand function.

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by
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PLASTICITY OF THE CORTICAL REPRESENTATION OF FINGER EXTENSORS INDUCED BY PAIRED ASSOCIATIVE STIMULATION

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CHAPTER 1

INTRODUCTION

1.1 Objective

In the United States, approximately 800,000 people annually experience a stroke (American Stroke Association). Stroke is the third leading cause of death in the United States and is the leading cause of major disability. With the emergence of quicker and more effective emergency care, the proportion of stroke survivors with major disability is rising as the stroke survival rate increases. Deficits in motor control affect a stroke survivors' capacity for independent living and economic self-sufficiency. The impact of even mild to moderate deficits in hand control in particular, affects numerous activities of daily living.

Effective rehabilitation of the hand is a significant challenge for several reasons. Foremost is the complexity of upper limb function. The upper limb is an interdependent system that requires the shoulder, elbow and hand to act in coordination with each other. The role of the upper limb is constantly changing from primary mover, to stabilizer, to manipulator as one interacts with an object, and this change is based on the physical, spatial and temporal characteristics of a task [19-21]. Another possible cause of this challenge is competitive neuromotor network plasticity. Cortical expression of hand and arm are adjacent and overlap somewhat with each other in the brain. A mutually inhibitory relationship between proximal and distal upper limb effectors in persons with stroke has been demonstrated experimentally [44]. Studies report that the repetitive practice of motor activities increases the area and density of cortical areas corresponding

to the practiced movement [126, 185]. This phenomenon of use dependent plasticity includes the sharing of overlapping cortical space with adjacent representations. Therefore, rehab training of arm prior to hand as traditional therapy might actually result in less cortical space for the hand to recover.

While there are numerous interventions aimed at enhancing recovery in the weakened upper limbs, functional outcomes are inconsistent [58, 111-113] and it is not clear whether these interventions actually improve recovery beyond inherent spontaneous resolution. Due to financial constraints, current rehabilitation models favor gait-training and proximal arm function [38]. And the effectiveness of intervention strategies have generally been less pronounced for the upper extremity than for the lower extremity [62-65, 73, 76]. Therefore, investigation into hand rehabilitation is an important topic in order to improve the potential outcome for survivors of stroke through recovery of skills of daily living.

Animal and human studies have shown that important variables in learning and relearning motor skills and in changing neural architecture are the quantity, duration and intensity of training sessions. There is evidence to demonstrate that plasticity is "use-dependent" and intensive massed and repeated practice may be necessary to modify neural organization [67-69] and affect recovery of functional motor skills [70-72]. The importance of intensity and repetition has also been confirmed for stroke patients in the chronic phase in the treatment paradigm referred to as constraint-induced movement-therapy (CIMT). Use-dependent cortical expansion has been shown up to 6 months after 12-days of CI therapy in people post stroke. In addition to the repetitive and intensive training necessary to induce neural plasticity, neuromotor stimulation must involve the

learning of new motor skills. Evidence strongly emphasizes that learning new motor skills is essential for inducing functional plasticity [38, 73]; therefore, it appears that critical variables necessary to promote motor changes and neural plasticity are the dynamic and adaptive development and formation of new motor skills.

Treatment protocols for patients with paretic upper extremities are labor intensive and require extensive one on one time with a physical therapist for several weeks and months. This impedance to eliminating functional limitations can be reduced by task-specific training that is repetitive, motivating, and augmented with feedback. Virtual reality technology may be an appropriate means to provide plasticity mediated therapies. Computerized systems are well suited to this and afford great precision in automatically adapting target difficulty based on individual subject's ongoing performance. Virtual environments can be used to present complex multimodal sensory information to the user and have been used in military training, entertainment simulations, surgical training, and training in spatial awareness and more recently as a therapeutic intervention for phobias. When virtual reality simulations are interfaced with movement tracking and sensing glove systems they provide an engaging, motivating and adaptable environment where the motion of the limb displayed in the virtual world is a replication of the motion produced in the real world by the subject. Our hypothesis for the use of virtual reality in rehabilitation post stroke is that this environment can monitor the specificity and frequency of visual feedback, and can provide graded rehabilitation activities that can be objectively and systematically manipulated to create individualized motor rehabilitation paradigms. Thus, it provides a rehabilitation tool that can be used to exploit the nervous systems' capacity for neuromotor adaptation. Previous studies have shown that patients

practicing in a VE have improved the kinematics of their hemiplegic hand function [5, 10, 11, 45-47]. We were able to track ongoing performance levels, use the data to precisely adapt the difficulty levels of the tasks to be learned and record precise kinematic and kinetic outcome measures on the patients' temporal and spatial components of hand motion during their training.

Changes in cortical excitability may be assessed using TMS. TMS has shown to be a noninvasive, painless and effective physiological assay of treatment-induced plasticity that can provide additional efficacy for using virtual environments for training motor recovery post-stroke. It has been demonstrated that by applying TMS to the motor areas while recording motor evoked potentials (MEPs) through electromyography, one can study the changes in neuromotor pathways post training. Single-pulse TMS is used to study the corticospinal pathways characteristics, such as amplitude, duration and onset latency of the MEP and to map the size of the cortical area representing a given movement. In stroke, this technique has demonstrated that restoration of strength and function is largely predicted by the integrity of the corticospinal tract system (i.e., lower MEP thresholds) and that the size of the cortical area representing the trained extremity is increased relative to the untrained extremity [13]. Using this technique the author investigated changes in MEP post-training and correlated these changes in excitability with the clinical behavioral measures.

There is an increasing interest in the use of brain stimulation to promote recovery of function post-stroke. Cortical stimulation can up or down regulate cortical excitability of both lesioned and non-lesioned hemispheres. It is believed that these changes in excitability can be used to facilitate re-learning and improved motor recovery. Although

poorly understood, the effects of cortical stimulation using TMS may be related to long-term potentiation or depression, and modulation of transmitter systems with changes in synaptic strength being the initial steps toward recovery of function [28]. Modulation of synaptic plasticity depends upon the timing of input and output on a neuronal level. A factor to consider is that TMS-induced changes in motor cortical excitability are usually evoked when the target muscles are relaxed: that is, there is no functional context for the change in cortical activity. A rarely studied relationship is the application of TMS during voluntary contraction. The hypothesis is that TMS applied during voluntary movements will strengthen neuronal networks associated with control of those movement patterns through long-term potentiation and synaptic efficiency. TMS, when synchronized with a specific movement has been found to enhance use-dependent reorganization in healthy volunteers [35] and improve manual performance when synchronized with maximal movement effort in the subject's post-stroke [74]. Here we investigated the clinical efficacy of using a TMS pulse time-locked to the initiation of the movement during the virtual reality training. Since this proved to be effective, it would serve as an add-on therapy to optimize training-induced plasticity in stroke subjects.

The mechanism of how the transfer of the skills acquired during the therapy translate to untrained movements is poorly understood. Deficits in the hand kinematics and inter-joint coordination of a hemiparetic arm have received some attention [23, 32, 38]. Hand kinematics of the affected arm are characterized by increased reaction time and movement duration, and decreased smoothness and accuracy. It is important to test whether changes in excitability and movement during the VR training will transfer to clinical function and non-trained natural hand movements. The degree of generalization

of the motor skills acquired during the VR training was tested in via industry standard functional tests. We measured whether there is an increase in the stability, accuracy and efficiency of these hand and arm functions as a result of PAS training. Fugl-Meyer, Wolf Motor Function Test were used to evaluate clinical changes as a result of PAS.

1.2 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive technique for stimulating the human brain by means of rapidly changing magnetic fields [13]. The stimulating effect is achieved by induction of brief cortical currents, which depolarize the cell membranes of both cortical excitatory pyramidal cells and inhibitory interneurons. If the depolarization exceeds a threshold level, the neuron will discharge. The effect of one TMS pulse can last up to a few hundred milliseconds. This TMS-evoked activity can be measured with a range of electrophysiological methods and several parameters of interest can be studied in the targeted network. The impact of TMS is determined not only by the properties of the stimulus, but also by the state of the activated brain region [8, 2, 86].

Long-term potentiation (LTP) is a long-lasting enhancement of synaptic communication, and is widely considered as a likely mechanism for the cellular basis of learning and memory [274, 22]. Bliss and colleagues [23] demonstrated *in vivo* in the rabbit hippocampus that field potentials of neurons in the dentate gyrus in response to single stimuli were increased following high frequency (from 10-100Hz), repetitive electrical stimulation of afferent projections to the dentate area. This increase in synaptic efficacy lasted for up to 10 hours in anaesthetized rabbits, and up to 16 weeks in anaesthetized animals. Further research has since shown that LTP is not a unitary

phenomenon and the mechanisms vary depending on the synapses and circuits in which they operate [145, 113, 114, 156]. Abundance of information from cellular level research as well as easy and effective accessibility of the motor cortex using TMS produced a great opportunity to translate synaptic level changes to the system level and the behavioral level using this technique. The motivation for this dissertation came from the preliminary studies in human showing promising diagnostic and therapeutic potentials for TMS assisted measurements and alterations of cortical excitability [204, 73].

TMS has been used for many different purposes including brain mapping and studying cortical reorganization and excitability [51]. TMS methodology has also widely used in patient studies, demonstrating excitability alterations in various diseases, including Parkinson's disease [272, 173], dystonia [246, 202], Huntington's disease [150], Tourette's syndrome [17], and essential tremor [36, 172].

1.3 Physiology of Motor-Evoked Potentials

Transcranial stimulation of the cerebral cortex to elicit motor-evoked potentials (MEPs) is a noninvasive method for assessing the integrity of the central motor pathway function. An MEP may be defined as the electrical muscular response elicited by artificially stimulating the motor cortex or motor pathway above the spinal motor neuron [276]. TMS was introduced in 1985 and since then has largely replaced the painful transcranial electrical stimulation (TES) [166] as a diagnostic clinical tool.

For routine MEP studies, the magnetic stimulator is connected to a standard EMG machine to synchronize the recording with the TMS pulse. Measuring MEPs from the upper limbs requires post-stimulus analysis time of fi50 ms, and 100 ms for the lower

limbs. If the CSP following the MEP is also analyzed, the recording time is typically extended to 300-500 ms [221]. MEPs are usually recorded with bipolar surface electrodes configuration taped to the skin overlying the target muscle. A low-pass filter of <1 Hz is recommended to minimize the duration of the stimulus artifact during magnetic stimulation [221].

The subject should be seated comfortably, with easy access to the subject's head and spine for stimulation of these areas. After localizing the optimal stimulation site, this coil position is usually marked with a pen on the scalp and used for the remainder of the testing for this muscle. The magnetic coil may be fixed with a coil holder or other stabilization device to ensure stable recordings without excessive coil movements.

Magnetic stimulators that are commercially available mainly induce two types of pulses 1) monophasic stimulator, with a rapid initial current and slow decays and 2) biphasic or polyphasic stimulator. Direction of induced current in monophasic stimulators depends on the coil's orientation while the biphasic stimulators are less dependent on the coil's orientation [31]. For most TMS studies and for more focal stimulation, 'figure-8' coils are used that consist of two adjacent round coils with opposite current direction. Mapping studies with a focal coil indicate that the distal upper-limb region on average best stimulated 5 cm lateral and 1-1.5 cm anterior to vertex and the proximal upper limb at 3.5-4 cm lateral and 0-0.5 cm anterior to vertex [277]. In another study, the optimal coil position for responses in a particular muscle varied up to 2 cm between individuals [168]. A useful approach in order to find proper stimulation spot is to stimulate at vertex and then 1 cm away in the four quadrants. Optimization of coil positioning is necessary for focal figure-8- coil because the MEP latency varies

significantly as the function of coil positioning [91]. In monophasic TMS current direction depends on coil orientation and largest responses are obtained when the coil axis is oriented 45-50 degrees to the parasagittal plane with a backward-owing current in the coil so that the induced current in the brain is perpendicular to the precentral gyrus owing posterior-anteriorly [31, 221].

MEP threshold is the lowest stimulus intensity of TMS that gives a recordable MEP in a target muscle. The motor threshold is usually provides a reference for setting the stimulation intensity for recording other parameters. A common definition of the MEP threshold at rest is the stimulus intensity required to elicit reproducible MEPs of 50 to100 micro-Volts in 50% of 10-20 consecutive trials [221]. It is practical to start the stimulation below the expected threshold intensity and increase stimulator output in a step up fashion with larger steps at values significantly lower than motor threshold and smaller steps in values close to the motor threshold until 50% of 10 stimulations produce a measurable response [208]. This method seems arbitrary and other techniques have developed to measure a more physiologically relevant motor threshold by defining two lower and upper thresholds. Lower threshold is the highest intensity evoking responses with a probability of zero and upper threshold is the lowest intensity that can produce MEP 100% of time. This method minimizes the number of stimuli needed. Measures of upper and lower thresholds are normally distributed and are independent of age, gender, and hemisphere [169].

MEP threshold is generally lower for distal than proximal muscles; lowest threshold values are reported for intrinsic hand muscles and finger extensors; this is consistent with their larger cortical representations of these muscle [221, 169]. Lower-

extremity muscles and pelvic muscles have higher thresholds. MEP threshold varies widely in the healthy population, with high correlation between siblings [277]. There is no consistent evidence to support a significant role of gender and age [169, 277]. A lower threshold has been reported for the dominant hemisphere [152, 265]. Other factors that have been shown to influence motor threshold are sodium-channel blockers [294] posture (lower when sitting vs. lying supine), mental activity [4] and closing and opening of eyes [223]. An inter-stimulus interval of >3 s has been recommended for determination of MEP threshold to prevent any facilitatory or inhibitory influence on the subsequent stimulation [42].

MEP latency can be defined as the time between the TMS and start of MEP recordings. MEP latency has been shown to be the most reliable (considering the inherent variability of measurements) of all the different parameters that can be measured by TMS induced MEP. MEP latency in combination with a measure of the peripheral nerve conduction time can produce the central motor conduction time which indicate the duration of central processing of the TMS evoked motor response and is a measure of pyramidal tract function.

MEP amplitude is another marker for the degree of cortical and pyramidal tract activation. Plus MEP amplitude may be a useful parameter of cortical excitability in combination of MEP threshold measurement [276]. MEP size can vary from stimulus to stimulus even when all the other stimulation parameters are kept constant [135]. Fast Fourier transformation and cross-correlation analysis did not identify a consistent dominant frequency for this variability, suggesting that the variability in MEP size could be random and maybe the result of varying synchronization, varying numbers of excited

motor neurons, or varying numbers of repetitive discharges however, the role of these factors are not clear. In one study, [77] wide range of variability in TMS induced compound MEP amplitudes in relaxed muscles was observed (coefficient of variation, range 0.22-1.12). In the same study, Ellaway and colleagues found a positive correlation for amplitudes of the MEPs in one muscle with those in the others. Clamping the coil relative to the head or altering the orientation of the coil all failed to affect the variability of MEPs [77]. This finding might suggest that variability in the MEP measures could stem from fluctuations in excitability of the corticospinal pathway. It is also possible that variability rise from small variations of facilitation by voluntary contraction or cognitive events.

MEP Variation may also stem from inadvertent movements of the coil during stimulation, even though previous studies have shown that the contribution of coil movements does not account for all of the observed MEP variability [77, 100]. Z'Graggen et al. [288] used triple stimulation technique with an additional nerve stimulus in the periphery to cancel the first descending action potential from TMS. This study showed a significant variability in repetitive motor neuron discharges after TMS however, further studies are necessary to confirm their findings [288].

1.4 Investigating Cortical Plasticity Using PAS Techniques

The human nervous system retains the potential for morphological and functional reorganization throughout life [232]. This potential for change has been termed plasticity. Plasticity of neural connections may occur at both the synaptic level [222] and at the regional level where changes can involve large networks of cells in response to lesions or

training [242, 214]. Plastic changes are believed to be the foundation for learning, memory and the repair of damage following brain injury [222].

Plastic changes occur in human motor cortex. One study showed that removal of sensory input can induce changes in cortical motor representation that reverse when the sensation was restored [104]. The mechanisms underlying cortical plasticity have been studied. These changes may be due to increased excitatory neurotransmitter release, increased density of postsynaptic receptors or the removal or reduction of tonic inhibition [40]. Reduced inhibitory inputs onto excitatory synapses is the most likely mechanism in short-term plastic changes and is likely due to reduction of GABAergic inhibition [40, 147]. This suggests that GABAergic neurons play a vital role in cortical map reorganization due to short term plasticity [125]. Another important process involved in short-term reorganization is the ability to modulate synaptic efficacy. Increased effectiveness of synaptic transmission was first described in the rabbit hippocampus [23, 24] where it was noted that stimulation of any of the three major input pathways resulted in increased amplitude of excitatory postsynaptic potentials in the target hippocampal neurons. This was termed long-term potentiation (LTP). It requires high frequency stimulation of excitatory afferents [24]; in contrast, low frequency stimulation can induce long-term depression (LTD) [71]. In general, the induction of LTP has four requirements: cooperativity, associativity, input-specificity and involvement of N-methyl-D-aspartate (NMDA) and GABA receptors [20, 22, 194, 200].

Cooperativity requires synchronous activation of neurons [24]. Associativity refers to convergent activity of pre and postsynaptic stimulation of neurons in a spike timing dependent pattern [20]. This is consistent with Hebb's postulate: 'When an axon of

cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased' [107].

The ability to form new synapses in the adult cortex is carefully balanced by the retraction of existing but perhaps unused synapses, so that the density of stable synapses remains unchanged [264]. Homeostatic regulation of neural circuits is necessary to prevent them from becoming hyper- or hypo-active [268]. In order to maintain this homeostasis, it is proposed that changes in synaptic weight, rather than wiring, may underlie cortical plasticity [44, 45]. However, a continuous increase in excitability cannot be maintained (limitation of Hebb's rule) within the physiologic range unless other compensatory or homeostatic mechanisms also modulate synaptic activities [268]. Bienenstock, Cooper and Munro in their mathematical model (BCM) proposed that the incoming patterns of impulses and change in the efficacy of a given synapse depends not only on instantaneous pre- and postsynaptic activities, but also on a slowly varying time averaged value of the postsynaptic activity [21].

Changes in afferent input can lead to a reduction of cortical inhibition. For example, withdrawal of sensory inputs has revealed rapid and dramatic alterations in the representational maps of M1 that mimic changes which occur following limb amputation [212, 297]. This is consistent with the view that the pattern of somatosensory input to the central nervous system plays an important role in maintaining cortical representation [32]. Conversely, relevant sensory stimulation can induce plastic changes that increase the representation of target muscles. Prolonged sensory stimulation, designed to mimic repetitive natural stimulation over a large skin surface, applied to adult owl monkeys

[126] resulted in significant remodeling of the primary somatosensory cortex, with considerable expansion of the stimulated receptive fields. Godde et al. (1996) extended this work, replacing repetitive nerve or digital stimulation with paired sensory inputs, according to Hebb's postulate [95]. This 'associative pairing' of tactile stimulation involved simultaneous weak electrical stimuli to two non-overlapping receptive fields of the digits of adult rats at random intervals. This resulted in enlargement of the stimulated receptive fields. A control experiment that stimulated only one skin site with the same temporal characteristics induced no change in receptive fields. A similar paradigm was then applied to human subjects and resulted in a significant improvement in spatial discrimination in the stimulated digits only [95]. This work forms the basis for the associative stimulation technique used by Ridding and co-workers [199].

1.5 Paired Associative Stimulation

An experimental paradigm widely used to induce plasticity in the human motor cortex is paired associative stimulation (PAS) [213, 250, 251]. This technique uses electrical mediated nerve or muscle stimulation paired with cortical stimulation. The electrical nerve stimulation and cortical TMS pulses are timed so that the peripheral input and the central stimulus arrive synchronously or near-synchronously at the motor cortex. The time between the two modes of stimulation is critical; initially 25 ms was chosen to allow for peripheral conduction time from the periphery to the somatosensory cortex (20 ms) and from there to the motor cortex (approximately 3 ms)**.** The effect of PAS on MEP size is noticeably dependent on the timing of the TMS pulse with respect to the afferent median nerve stimulation. Stefan et al. [251] discovered that interstimulus intervals up to

35 ms were effective in generating LTP-like effect, provided the peripheral volley arrived prior to the cortical stimulus. Reversing the sequence of arrival of the afferent signals so that the peripheral volley arrived after the cortical stimulus induced depression of cortical excitability, as proposed by the strict temporal Hebbian rules [285]. This is consistent with the idea that induction of plasticity in this way is similar to LTP and LTD in spike timing dependent paradigm. Repetitive stimulation of either the periphery or the cortex, while not strictly fulfilling the requirements for associative LTP plasticity, may also induce plastic change in the somatosensory cortex. Prolonged peripheral nerve stimulation [130, 37, 138, 287], muscle vibration [218] or high frequency stimulation of the motor cortex with repetitive TMS (rTMS) (greater than 5 Hz) [192] also result in enhanced cortical excitability of the target muscles. In contrast, low-frequency rTMS (1Hz or less) may depress motor cortical excitability [39].

Evidence suggests that the site of action of PAS-induced plasticity is at the level of the cortex: Apart from increasing the size of the MEP amplitude, PAS led to an increase in the duration of the silent period recorded from the pre-contracted muscle. This observation points to a cortical site of the PAS-induced plasticity as the silent period is generated cortically [250]. Electrical brainstem stimulation, which excites corticospinal axons directly at the level of the cranio-cervical junction downstream of the cortex [270], remains unchanged after PAS [251]. Also, the F-wave which is an index of spinal motor neuron excitability, does not change after PAS [251]. Finally, PAS interferes in a highly specific manner with volitional preparatory cortical motor activity, as measured by changes in movement-related cortical potentials (MR-CPs) in EEG recordings. PAS affects MRCPs only of those movements targeted by PAS.

PAS is capable of producing both LTP and LTD-like plasticity. This bidirectional effect depends on the timing between the pairs of stimuli. Therefore it has been suggested that PAS is a type of spike timing dependent plasticity (STDP) [171]. Among the few properties of PAS technique are its rapid induction (after intervention of only 30 min), long duration, reversibility, and NMDA-receptor activation. After facilitatory PAS (ISI of 25ms), MEP-amplitudes increased for at least 60 min. After inhibitory PAS, MEP amplitudes remained depressed for approximately 120 min. The changes in cortical excitability reversed within 24 hr after PAS25 [251]. Both the increase and the decrease of MEP amplitudes following facilitatory PAS or inhibitory PAS were blocked with dextromethorphan, an NMDA receptor antagonist. Moreover, PAS10 failed to induce a decrease in MEP size if the subjects were pre-medicated by nimodipine, an L-type voltage gated calcium-channel antagonist. These features indicate that the mechanism behind PAS probably occurs through synaptic modification and fits with the spike timing dependent plasticity model.

PAS can induce a somatotopically specific plasticity. In one study, both APB and FDI were stimulated by TMS however, choosing median nerve for peripheral nerve stimulation (APB is innervated by Median nerve) the amplitudes of TMS-evoked MEP recorded from the first dorsal interosseous muscle (FDI innervated by Ulnar nerve) remained unchanged in the presence of a substantial increase in the MEP amplitude recorded from the APB muscle, which had the central representation stimulated by PAS [213]. Other studies have also found that the effect of the PAS25 was specific to the hand area and recording from muscles in upper arm and foot did not show any facilitation after the stimulation [251, 279].

Similar techniques can also be applied to other brain networks to study plasticity and integration of sensory stimuli in cortex. For example, the PAS technique was used to induce plasticity in somatosensory cortex. PAS was applied to the primary somatosensory cortex by repetitive stimulation of the median nerve stimulation followed by TMS targeted to the somatosensory cortex. This procedure led to significant enhancement of the amplitude of the P25 of somatosensory evoked potentials (SEP) obtained from median nerve stimulation. Similar to motor cortex, the relative timing of the stimulation modalities was critical for modulation of SEP and plasticity induction were bidirectional in nature [142]. Network specificity and other features of the PAS technique described above made this a suitable technique to investigate features of motor control and motor learning mechanisms in human subjects.

1.6 Movement Related Cortical Stimulation

In previous forms of PAS, the peripheral electrical stimulation induces activity in M1 through thalamocortical "vertical" and/or corticocortical fibers from the somatosensory cortex [285]. However, it has been rarely investigated whether other types of afferent input to M1 combined with TMS can produce similar associative LTP-like effects or not [81, 245]. One recent animal study showed that the repetitive activation of the artificial connection between M1 neurons via implantable electronic circuits can produce long-term plasticity [93]. If associative stimulation is a general principle for human neural plasticity, it is possible that natural physiological activation of M1 during the reaction time task synchronized with TMS can also produce associative LTP/LTD-like plasticity.

In this dissertation instead of pairing just peripheral stimulation [250-1, 285] or contralateral M1 stimulation [82, 203] with TMS, we paired voluntary finger extension with TMS over M1 and electrical stimulation of the ED; movement-related paired associative stimulation (MRPAS). We hypothesized that MRPAS combining PAS with endogenous movement-related activity in M1 can induce timing-dependent plasticity in motor function.

1.7 Clinical Applications of PAS

PAS can provide a unique perspective to study disorders of plasticity. The capability to produce LTP, reproducibility, and network specificity can be used to investigate the pathophysiology of neurological disorders. One disorder in which neuro-plasticity has been suggested to play a pathogenic role is focal dystonia, which occurs in some subjects with repetitive movements. Several studies have revealed that neuronal representations are altered in focal hand dystonia. Digit somatotopy and inter-digit spacing are altered and these changes may be linked to repetitive actions and neuroplasticity [177, 201, 202].

Quartarone first described increased cortical response to PAS in patients with focal hand dystonia, showing that neuroplasticity is disturbed in patients with writers' cramp, a form of focal hand dystonia [200]. Other studies then discovered plastic changes in digit representation in cortex as well as abnormal homeostatic mechanisms in stroke patients [177, 202]. Furthermore, patients with focal hand dystonia lacked the normal increase in silent period duration induced by PAS, a physiological measure that has been linked with neuronal inhibition mediated by GABA -receptors. This finding confirms that abnormal neuronal plasticity play a role in pathophysiology of focal hand dystonia.

PAS has also been used to investigate plasticity in other diseases. Levodopa induced dyskinesia, which is related to the drug treatment in Parkinson's disease, has also been associated with aberrant plasticity in the human motor cortex (M1). PAS induced LTP was shown to be deficient in Parkinson's disease off medications and was restored by levodopa in non-dyskinetic subjects. However, a deficient plastic response remained in patients with dyskinesia [173]. Reduced LTP-like effects have also been seen in those affected by stroke [52]. PAS can modulate the human sensorimotor cortex in predictable and bidirectional pattern. This promising protocol may offer a tool to investigate the mechanisms of cortical plasticity in humans. It provides us with a tool to modulate as well as to detect abnormal cortical plasticity. In this dissertation, we sought to establish the exact parameters for PAS as a useful intervention for rehabilitation of the hand extensor muscles after stroke.

1.8 Dissertation Aims and Hypotheses

Plasticity is one of the foundational functional blocks of our nervous system. In this dissertation, we investigate ways to induce, modulate and alter cortical excitability using rules of associative plasticity. Our primary goal was to find a reproducible, effective, simple and physiologically meaningful method to improve adaptation and motor skill learning in human subjects using associative plasticity rules to non-invasively stimulate the human motor cortex. The specific hypotheses of this dissertation include:

1. PAS improves human motor function in stroke subjects through increasing the weight of synapses in sensory motor network.

2. PAS increases rapidity of movement and improves clinical function of the hand.

3. Visuomotor feedback supplied by VR can affect the PAS-LTP like effect and influence motor learning.

4. ISI values >20 ms will increase PAS mediated effects in the ED in both healthy subjects and in those with neurological impairment.

5. The effects of this PAS paradigm will be specific to the target muscle relative to paradigms that target the intrinsic hand muscles.

These hypotheses were transferred into three specific aims as follows:

Aim 1a

To further develop an effective virtual reality (VR) based paired associative stimulation (PAS) platform that allows for the determination of the optimal stimulation and behavioral parameters for PAS induced LTP in the finger extensors. Here the goal was to integrate our existing VR environments and TMS system to design a protocol in which subjects could recognize virtual feedback of their hands and use hand kinematics to drive and then test PAS. The author investigated whether or not adjusting the motor behavior and stimulation parameters to maximize corticomotor excitation as subjects performed simple finger extension movements.

Aim 1b

To investigate the specificity of PAS-LTP like effects in the extensor digitorum and primary motor cortex in healthy subjects. The goal here was to provide a measure of corticomotor excitability and determine how specific the desired excitatory effects of PAS are relative to the target muscle. Given previous results showing widespread and contradictory results in muscles when the nerve is directly stimulated in the intrinsic hand

muscles, we predicted that stimulation delivered directly to the muscle belly of an extrinsic hand muscle (ED) would be more specific in its modulation of M1 excitability.

Aim 2a

To investigate the post-training effects of VR based PAS training on corticomotor excitability in healthy individuals when interstimulus interval (ISI) is increased and the effect of using EMG activity to trigger stimulation. The goal here is to test if longer ISIs will increase the facilitatory effect of PAS in the finger extensors. Given the time sensitive polarity of the LTP/LTD effect and the location of the extensor digitorum, we predicted that a longer ISI of 25 ms will further augment M1 excitability.

Aim 2b

To investigate the post-training effects of VR based PAS training on corticomotor excitability when electromyography (EMG) is used to trigger the paired stimulation. The goal here was to use the EMG activity of the target muscle to initiate the paired stimulation during training instead of finger movement. Given the variability in hand size and range of motion in stroke patients, and that the neuromechanical delay inherent to muscle dictates that EMG activity is initiated prior to finger movement, we predicted that stimulation earlier in the movement would modulate corticomotor excitability to a greater degree than movement triggered stimulation.

Aim 3

To investigate the post training effects of EMG driven paired associative stimulation on primary motor cortex excitability in patients with stroke. The goal here was to take the optimal PAS parameters established in Aims 1 and 2 and determine if the PAS effects in stroke patients mirror the effects seen in healthy subjects. The results from Aim 1 and 2 led us to predict that longer ISIs and EMG driven stimulation would increase corticomotor excitability and also allow for detectable changes in hand function. Ultimately, this knowledge will allow us to develop effective stroke rehabilitation paradigms.

CHAPTER 2

EFFECT OF BEHAVIOR AND STIMULUS ON EXCITABILITY

2.1 Abstract

It has been shown extensively in the intrinsic hand muscles of healthy subjects that paired associative stimulation (PAS) combining peripheral electrical stimulation and transcranial magnetic stimulation (TMS) induces lasting changes in cortical motor excitability. However, there is a dearth of investigations to determine what the optimal parameters for PAS are regarding the extrinsic muscles of the hand in healthy subjects. This study attempts to identify the ideal conditions for facilitating changes in excitability in the extensor digitorum of healthy subjects. Once established, these parameters could be employed in the area of neurorehabilitation. Because the motor recovery of the distal upper limb and particularly finger extension is a major challenge to rehabilitation, we investigated the effect of PAS on the excitability of the corticospinal projection to the extensor digitorum (ED) muscle as measured by motor evoked potential amplitude before and after PAS in 21 healthy subjects. The topographical specificity, the effects of stimulation type (single pulse vs. train), inter-stimulus interval (ISI, 20 ms vs < 20 ms), and the respective role of cutaneous and muscular afferents (movement vs. rest) in facilitating motor excitability were also studied. Using several protocols under varying motor and stimulation conditions, PAS was able to induce changes in the excitability of corticospinal projection to the finger extensor muscles in healthy subjects. The electrophysiological features of these changes were similar to those previously observed in intrinsic hand muscles: quick progression (present after just 30 minutes of training), topographical specificity (limited to the target muscle) and associative dependence

(interstimulus intervals < 20 ms failed to elicit excitatory effects) suggesting an LTP-like mechanism. When combined with volitional movement generated afferents, the effect on M1 was significantly larger compared to when PAS was performed at rest. Consistent and repeated PAS protocols showing excitability changes in the ED help to confirm that the movement single pulse technique could be most relevant in motor rehabilitation for some stroke patients. A second study in stroke subjects examining excitability and functional improvements was conducted and will confirm this effect is applicable to an impaired population.

2.2 Introduction

Paired Associative Stimulation (PAS) has achieved distinction as a potential rehabilitative intervention for the treatment of neurological injury and disease. PAS is a valuable tool with which to examine Hebbian principles of neural plasticity in humans. Hebb's postulate states that When an axon of cell *A* is near enough to excite cell *B* or repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that *A*'s efficiency, as one of the cells firing *B*, is increased. Through PAS two signals (afferent and efferent) arrive simultaneously at M1 in order to facilitate this Hebbian mechanism. Increases in the cortical response after PAS support the idea that joint activity of the synaptic units leads to a strengthening of synaptic efficiency. Prototypically, a single electrical stimulus is directed to a peripheral nerve in advance of transcranial magnetic stimulation (TMS) delivered to the contralateral primary motor cortex (M1). Repeated pairing of the stimuli (i.e., association) over an extended period may increase or decrease the excitability of

corticospinal projections from M1, in manner that depends on the interstimulus interval (ISI). It has been suggested that these effects represent a form of associative long-term potentiation (LTP) and depression (LTD) that bears resemblance to spike-timing dependent plasticity (STDP) as it has been elaborated in animal models. Paired associative stimulation (PAS) combining peripheral electrical stimulation and transcranial magnetic stimulation (TMS) induces long term plasticity like changes in the corticospinal projection to hand muscles in normal subjects [251]. This procedure allows for the study of Hebbian-like mechanisms of synaptic plasticity in the human motor cortex. If a weak excitatory input (afferent peripheral electrical stimulation) triggered 20 ms prior to a TMS pulse given over the target muscle area of the contralateral motor cortex, repeatedly arrives at cortical level, then a single pulse TMS of the target muscle area evokes a larger motor evoked potential (MEP) than before PAS. The mechanism responsible for this change remains unidentified but it is hypothesized that a form of long-term-potentiation (LTP) may contribute to this induced associative plasticity [250, 252, 285]. Paired stimulation combining motor point stimulation of the first dorsal interosseous (FDI) muscle and TMS on three successive days was able to induce long-lasting reorganization of the cortical representation of the target muscle which lasted for at least 2 days following the last stimulation session (McKay et al. 2002). PAS protocols utilizing direct muscle stimulation have also been successful in producing LTP-like effects such as changes in intracortical excitatory circuits [164, 199].

PAS repeatedly pairs electrical stimulation of a peripheral nerve with TMS of the contralateral sensory or motor cortex at a constant interstimulus interval (ISI). The ISI between the peripheral electrical stimulation and the TMS pulse plays a crucial role in

defining the polarity of the effects on corticospinal excitability [153-155, 189, 265]. ISIs below 20 ms have given rise to inhibitory effects on upper limb muscles while those greater than 20 ms lead to excitatory changes in M1 [38]. These observations suggest that determining the requirements for excitability changes induced by PAS protocols in normal motor cortex may be relevant for the rehabilitation of patients with neurological injury. PAS literature on the upper limb largely investigates finger abductor or interosseous muscles [249-252, 273]. The extensors of the upper limb are less often examined [38, 161]. We studied finger extensor muscles because the motor recovery of distal upper limb in post-stroke patients is a significant challenge to rehabilitation and is of particular importance in recovery of autonomy lost after stroke.

The current literature indicates that the effects of excitatory PAS are not necessarily limited to the muscles innervated nerve receiving electrical stimulation [38]. There are reported instances in which changes in the excitability of corticomotor projections induced by classic PAS protocols have been more pronounced for muscles that are innervated by a different nerve [59]. In response to the finding that excitatory effects in the ulnar nerve innervated ADM that could not be distinguished from those obtained in the median nerve innervated APB. This has been referred to as a "somatotopic gradient." [163, 251]. The term "topographical specificity" [132, 201] has been used to imply that alterations in excitability brought about by PAS are restricted to the cortical representations of muscles innervated by the peripheral nerve that was stimulated electrically [251]. In this study, we assessed the degree to which the notion of topographical (i.e., muscle) specificity applies to this method of PAS. This study shows

that specificity of the excitatory effects is present when the target muscle is the site of electrical stimulation.

In many studies in which PAS protocols are employed, EMG recordings are obtained only from a single (target) muscle. This is typically the ulnar nerve innervated abductor digiti minimi (ADM), the median nerve innervated abductor pollicis brevis (APB), or the ulnar nerve innervated first dorsal interosseus (FDI). In some cases, however, potentials evoked in other muscles are recorded prior to and following the administration of PAS. For example, in the study by Stefan et al. (2000), the median nerve was stimulated electrically, and although APB was the muscle of interest, MEPs were also recorded the musculocutaneous nerve innervated biceps brachii (BB) muscle, though they were of a much lower magnitude. Using a similar intervention, Rosenkranz and Rothwell (2006) found that for healthy subjects, increases MEP amplitudes recorded in the ulnar nerve innervated FDI were of similar size to those obtained for the (target) APB [251]. In cases in which the changes in the excitability of corticospinal projections to non-target muscles have not been statistically consistent, the effects have been in the same direction as those induced in the target muscle [52, 200-202, 278, 280].

In previous forms of PAS, the peripheral electrical stimulation induces activity in M1 through corticocortical fibers from the somatosensory cortex [280]. However, it has been rarely investigated whether other types of afferent input to M1 combined with TMS techniques can produce similar associative LTP-like effects or not [81, 155]. Studies combining voluntary movement and TMS or movement and PAS have been performed on the lower extremities; others have used exercise prior to PAS in order to prime the excitatory effects on the upper limb [162, 245, 252]. These studies have framed the PAS

effect in humans as similar to spike-timing-dependent plasticity, due to the dependence on the polarity of the effect (excitatory, inhibitory) on the order of the stimuli. It remains the case however, that few empirical studies have combined PAS and voluntary movement to scrutinize the role of other mechanisms in the facilitation of excitability.

In the present study, our scope was twofold. First we present the design of we investigated the effect of PAS application during relaxation and voluntary muscle contraction on motor cortical excitability. Second we examine the effect of stimulus type on excitability. We sought to test directly whether movement and stimulus type affects corticomotor excitability, by studying MEP amplitude changes after PAS training. We measured MEPs with the assumption that any change in the corticospinal excitability following PAS represent and excitability change at the level of the primary motor cortex. We investigated the effect of one session of paired associative stimulation on the excitability of the corticospinal projection to extensor digitorum (ED) muscle (MEP amplitude before and after PAS) in healthy subjects. We also sought to establish the topographical specificity, the effects of stimulation type (single pulse vs. train), inter-stimulus, and the respective role of cutaneous and muscular afferents (movement vs. rest) in facilitating motor excitability. Our published and preliminary data suggest that our virtual reality (VR) environments provoke a sense of reality to the subjects, provide valid and reliable measures of hand kinematics, and possess potential as a rehabilitation tool in clinical populations [6, 7, 17]. Our preliminary data also established that interstimulus intervals lower than 20 ms were unable to affect significant increases in M1 excitability so 20 ms was chosen as the ISI across all four protocols. It will be shown that our PAS protocol adapted from the methods used by Ridding and Taylor (2003) was able to induce

significant changes in excitability of the extensor digitorum motor cortex area in healthy subjects and that an movement-single pulse design will be optimal for use in the rehabilitation of the hand post-stroke [164].

2.2 Methods

2.2.1 Subjects

Twenty-one right-handed healthy individuals (14 male, 7 female; age 22-32 years) volunteered after giving their written informed consent to the study approved by the Rutgers and NJIT institutional review boards. Subjects attended on two to four occasions in a within-subjects study design. One or two of four possible interventions: (1) Movement Train PAS (2) Rest Train PAS (3) Movement Single pulse PAS and (4) Rest single pulse PAS were delivered at each visit, separated by 60 to 90 minutes. Each PAS protocol was randomly assigned to the visit.

2.2.2 Procedure

The subject was seated with their right arm supported at the hand, wrist and elbow in a custom built armrest. The wrist was slightly flexed and the apparatus allowed for 90º of finger extension/flexion. The forearm was positioned partially supine on a curved, padded rest so that the ulnar styloid sat just on the edge of the padding. Their arm was positioned such that the subject's arms were aligned with a pair of onscreen virtual hands created using Virtools Software (Dassault Systems) on a TV display. The fingers were placed in a relaxed, flexed position at the MCP, PIP and DIP joints. To maximize the perception of realness, the TV was positioned horizontally above the hands and angled so

that the vantage point of the virtual hands, which was driven by glove data, corresponded to the subject's actual hands underneath the TV (Figure 2.1). Our published data suggest that this setup provides a sense of ownership of the virtual hands and we have successfully employed the virtual reality hand feedback in a number of healthy and patient-based studies [6, 7, 17].

The four conditions are described below. For all conditions, subjects viewed a virtual reality environment with two virtual hands. Subjects were asked to watch the screen and focus their attention to the moving hand. During the movement conditions, text commands 'OPEN' or 'CLOSE' were displayed and trials were initiated every 4-6 seconds (duration was random to minimize predictability) to cue hand movement.

2.2.3 Rest PAS

The text commands OPEN and CLOSE were covered up and the subjects simply observed their motionless virtual hands. Each time the OPEN command was displayed (but not seen by the subject) Stimulation was automatically delivered. Paired stimulation was given with either a single pulse (Rest-single pulse) or train (Rest-train) of electrical stimulations, followed by TMS delivery. PAS was applied every 4-6 seconds (duration was random) and continued for 30 minutes for a total of 250 paired stimulations.

2.2.4 Movement PAS

Subject position was identical to Rest PAS but the target hand initial position was recorded by a Cyberglove (CyberGlove Systems) wired 22 sensor data glove. All hand movements were recorded by the glove which was calibrated for each subject

(VirtualHand Software, CyberGlove Systems). Once calibrated, the subject's hand movements were shown in real time on the display. Each subject was asked to follow the onscreen commands. Subjects were given instructions to open and close their hand at their normal rate. On screen targets were used to prevent hyperextension of the fingers. PAS stimulus was triggered by a 25º change from the resting MCP joint angle. Duration of PAS, interval between stimuli and total stimuli delivered was the same as Rest PAS. Paired stimulation was given with either a single pulse (Movement Single pulse) or train (Movement Train) of electrical stimulation, followed by TMS delivery. PAS was applied every 4-6 seconds (duration was random) and continued for 30 minutes for a total of 250 paired stimulations.

Figure 2.1 Top view and side view of the forearm and hand position in the armrest along with the subject's view of the virtual hands controlled in the experiment.

2.2.5 Electromyography

Surface electromyographic (EMG) activity was recorded from five wireless Trigno™ electrodes (Delsys Inc.) placed over the muscle belly of the right extensor digitorum, right flexor digitorum, right flexor digitorum indicis, right extensor indicis and the abductor digit minimi. EMG signals were amplified and band-pass filtered before being digitized at 1000 Hz. EMG was recorded continuously and then processed with each MEP comprising a window spanning 50 ms prior and 100 ms after stimulation using a custom built MATLAB acquisition and analysis system (Mathworks Inc.).

2.2.6 Neuronavigated Magnetic Stimulation

Single-pulse TMS (Magstim Rapid2, 70mm double AFC coil) was applied at 110% of the resting motor threshold, the minimum intensity required to elicit MEPs > 50 μV in the right extensor digitorum (ED) muscle in 4 out of 6 consecutive trials. For subjects who had undergone MRI, a high-resolution anatomical MRI scan (3T Siemens Allegra) was used to render a 3-D cortical surface. A visor with motion tracing markers was placed on the subjects head. Fiducial locations on the MRI were core-registered with the subjects head to allow frameless neuronavigation (Visor, Advanced Neuro Technology). The optimal site of stimulation for ED (i.e., the hotspot), determined from initial exploration, was defined as the site with the largest MEPs for a given supra-threshold stimulus intensity, and used throughout the experiment. The stimulated ED hotspot of the motor cortex was marked on the MRI scan. The coil was held tangentially with the handle facing 45º posteriorly off the sagittal plane, and was tracked online to be stay over the ED

hotspot. For subjects without an anatomical MRI, their head was co-registered to a model MRI.

During PAS, TMS was triggered 20ms after peripheral electrical stimulation was delivered to ensure both signals arrived at the cortex simultaneously. 250 TMS pulses were delivered to the ED hotspot at 110% RMT with a frequency of approximately 0.14 Hz. The level of attention, a significant modulator of PAS-LTP effects [252], was controlled and attention was maximized to the simulated hand by a color change on the virtual display when the command changed. For all four conditions the subjects were asked to count and report the total number of stimuli they received as correctly as they could at the end of PAS.

The interventional paired stimulation was performed with electrical stimulation of the target muscle (innervated by the radial nerve) by placing the bipolar electrodes just proximal to the muscle belly of the ED. Intensity was chosen as 110% of that sufficient to produce a just noticeable twitch in the ED muscle at rest (8.8 ± 1.3 mA, 300 V_{max}, n =21). Stimulus was performed with a Digitimer DS7A stimulator (Digitimer Ltd.) using constant current square wave pulses (cathode proximal, stimulation width 1 ms) followed 20ms later by TMS. Single pulse PAS consisted of 250 stimulus pairs at a frequency of 0.2 Hz. Train PAS consisted of trains of 500 ms duration consisting of 1 ms square waves delivered at 10 Hz (i.e., 5 stimuli per train) with TMS delivered 20ms after the last shock of the train.

2.2.7 Experimental Design

Each subject was randomly assigned to a PAS treatment order (Figure 2.2). Time between sessions varied from 1-12 days. Forty MEPs (using 110% RMT) were collected pre and post intervention over the course of 4 minutes. To maintain consistent EMG activity across trials and conditions, subjects were asked to relax their hands (monitored by real-time EMG) in the neutral start position when not opening or closing their hand. The time between treatments on the same day was a minimum of 60 minutes to allow for sufficient washout of any previous PAS effects [252].

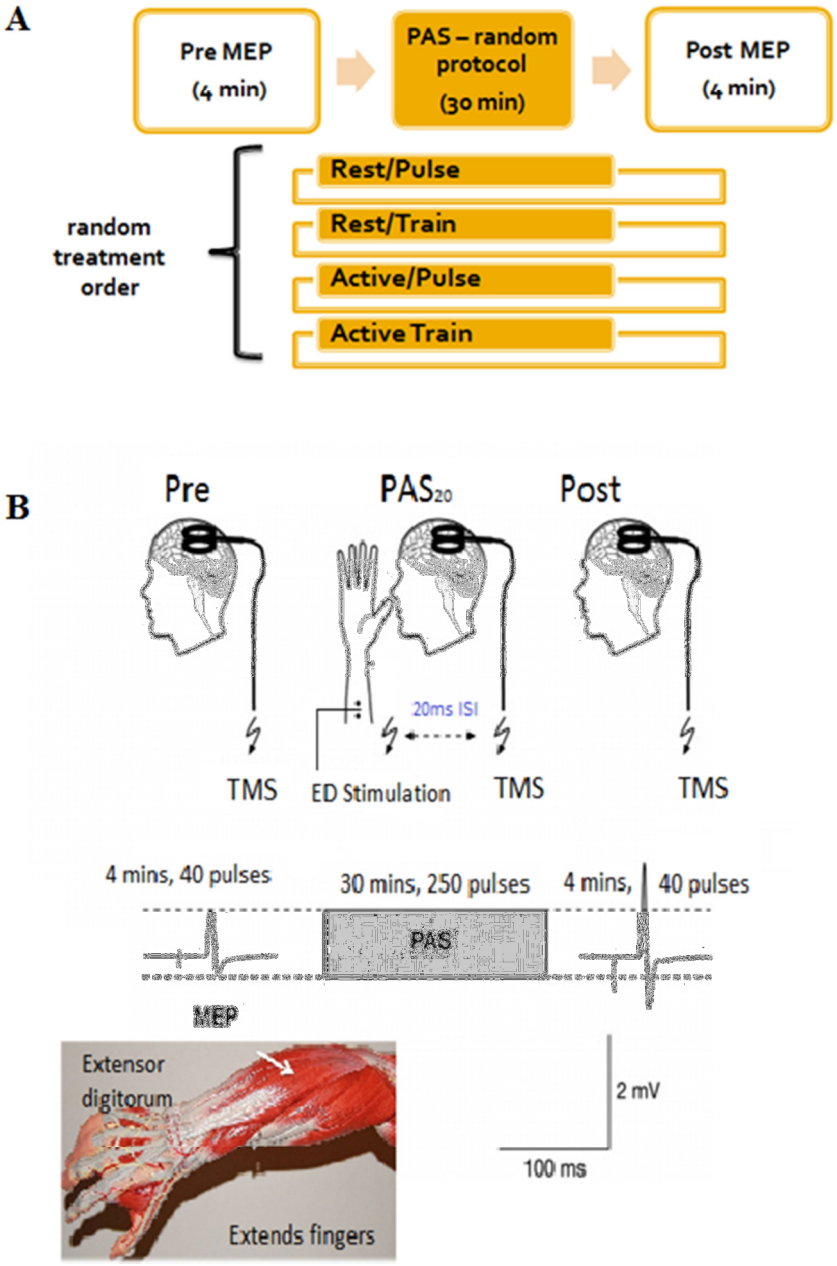

Figure 2.2 Schedule and Design of study 1. A: The four PAS protocols. In the main experiment, motor-evoked potentials (MEPs) were recorded before, during and after intervention with the stimulation intensity that evoked MEPs of, on average, 1mV peak-to-peak amplitude in the resting extensor digitorum at baseline. Note that subjects took part in four experimental sessions in a crossover design with different intervals between the consecutive sessions of two identical LTP-like plasticity inducing PAS protocols. B: Target muscle and exact PAS protocol used (ISI varied along with movement requirement).

2.2.8 Statistics

Electrophysiological variables (MEP, EMG) were averaged across trials for each condition and subject. Means were submitted to a repeated-measures analysis of variance (rmANOVA). Four paired t-tests were used to compare pre and post PAS MEP amplitudes across all four protocols (using a Bonferonni correction). Finally, rmANOVA was also performed for to characterize the degree to which each condition (movement, stimulation type) contributed to the MEP excitatory effect. For this, MEP was defined as the dependent variable stimulation type and behavior (movement, rest) as independent variables. Data were analyzed with PASW Statistics 18 (SPSS). rmANOVA was used to test for main effects and interactions. Statistically significant interaction effects were tested post hoc by Tukey's honestly significant difference (HSD) test. Significance threshold was set at $P < 0.05$.

2.3 Results

The mean peak-to-peak amplitude MEPs increased significantly for each protocol pre vs. post. The post-PAS data was grouped by intervention type (movement and single pulse; rest and single pulse; movement and train; rest and train) and referred to as Movement single pulse, Movement train, Rest single pulse and Rest train, respectively. The mean MEP amplitude for each group was expressed as a ratio to mean pre-intervention amplitude. Group mean data post-PAS was tested for normality, and then compared to pre-PAS baseline using a 2x2 ANOVA.

To test the immediate effect of active movement on MEP amplitude relative to the resting protocol, the mean of approximately 40 post MEPs was examined with

respect to pre-intervention using repeated measures ANOVA, Data are presented as mean ± SEM. 'Normalized' data refers to expression as a ratio to pre-intervention baseline. The results show that behavior does have a significant ($p < 0.05$) effect on PAS-LTP like effects. This suggests that active protocols should be used for interventions with stroke subjects to maximize rehabilitation potential related to plasticity and excitability changes.

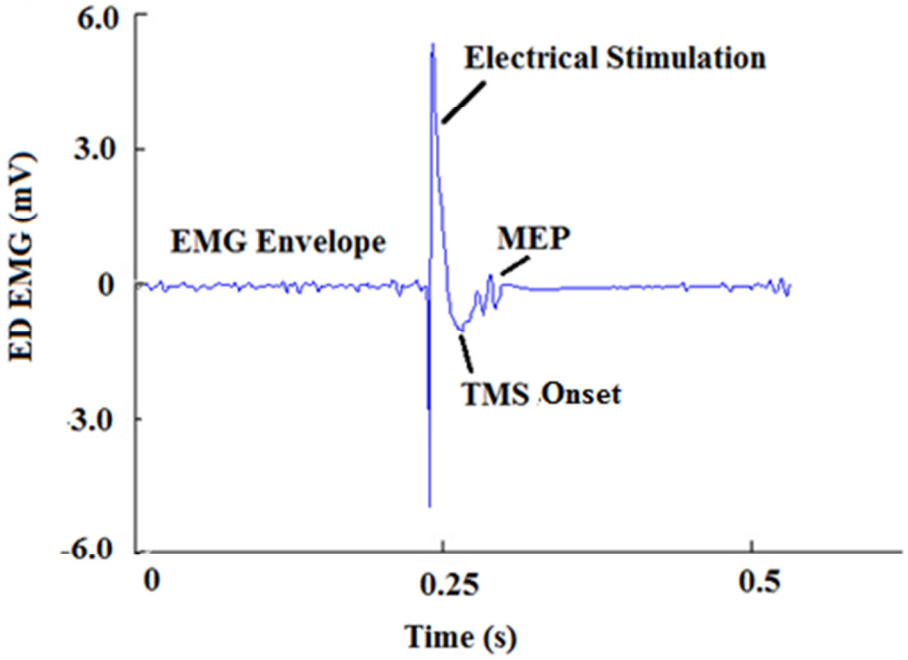

Figure 2.3 Raw and filtered/rectified (thin line) EMG signal acquired from a typical subject in experiment PAS_{20}. ED, extensor digitorum; TMS, transcranial magnetic stimulation; MEP, Motor evoked potential.

A statistically significant increase in the MEP amplitude was observed after the PAS intervention, but not just for movement PAS interventions (Figures 2.4 and 2.5). Data showing representative sample resting MEP waveforms for one subject at baseline and post-intervention for Movement single pulse, Rest single pulse, Movement train and Rest train protocols reveals post PAS intervention MEP amplitude (mV) was

significantly increased in all four conditions: Movement single pulse = 0.61 ± 0.04 (141 ± 25%, P < 0.05); Rest Pulse = 0.54 ± 0.03 (119 ± 14%, P < 0.05); Movement train = 0.57 ± 0.05 (124 ± 25%, P < 0.05; Rest train = 0.52 ± 0.04 (118 ± 21%, P < 0.05). Following the Rest train and Rest Single pulse intervention, however, the elevation in mean MEP amplitude was lower than in the movement conditions. rmANOVA analysis demonstrates that the motor activity during PAS had a significant effect on excitation (F (1,20) =8.7, p = 0.008).

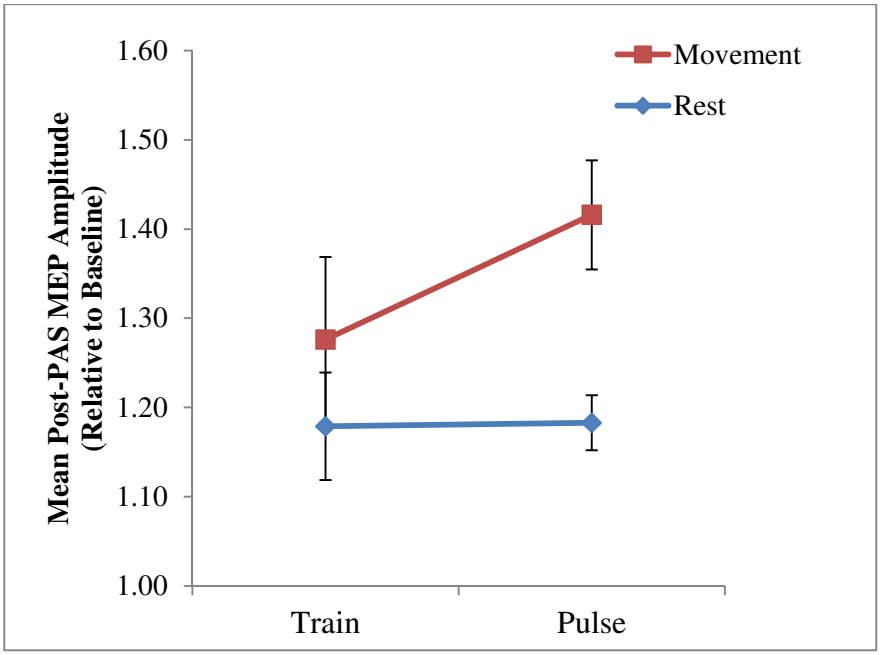

Figure 2.4 2x2 repeated measures ANOVA was performed to determine if behavior (movement vs. rest) during PAS intervention had an impact on MEP amplitude. The results show that behavior does have a significant ($p < 0.05$) effect on PAS-LTP like effects. This suggests that movement involved protocols should be used for interventions with stroke subjects to maximize rehabilitation potential related to plasticity and excitability changes.

The results showed that single pulse stimulation protocols resulted in greater increases in excitability as measured by MEP amplitude when compared to rest protocols (Figure 2.7). A 2x2 repeated measures ANOVA determined that the electrical stimulus type (single pulse or train train) used during PAS had a significant impact on the

excitatory effect on MEP amplitude ($F(1,20) = 11.24$, $p = 0.003$). The results show that single pulse stimulus has the greater excitatory effect on M1 compared to the train stimulation. The interaction effect with both motor activity and stimulation type as also found to be significant ($F(1,20) = 6.01$, $p = 0.023$).

In this study, analysis of the additional muscles (FD, FDI, EI ADM) recorded showed no significant changes in MEPs obtained (Figure 2.8). Only the control muscle, the ADM, failed to produce any MEPs throughout the experiment. The other non-target muscles all showed highly variable responses and these effects were not consistent across the four PAS protocols. Analysis performed on these muscles shows no significant changes in MEP amplitude after PAS.

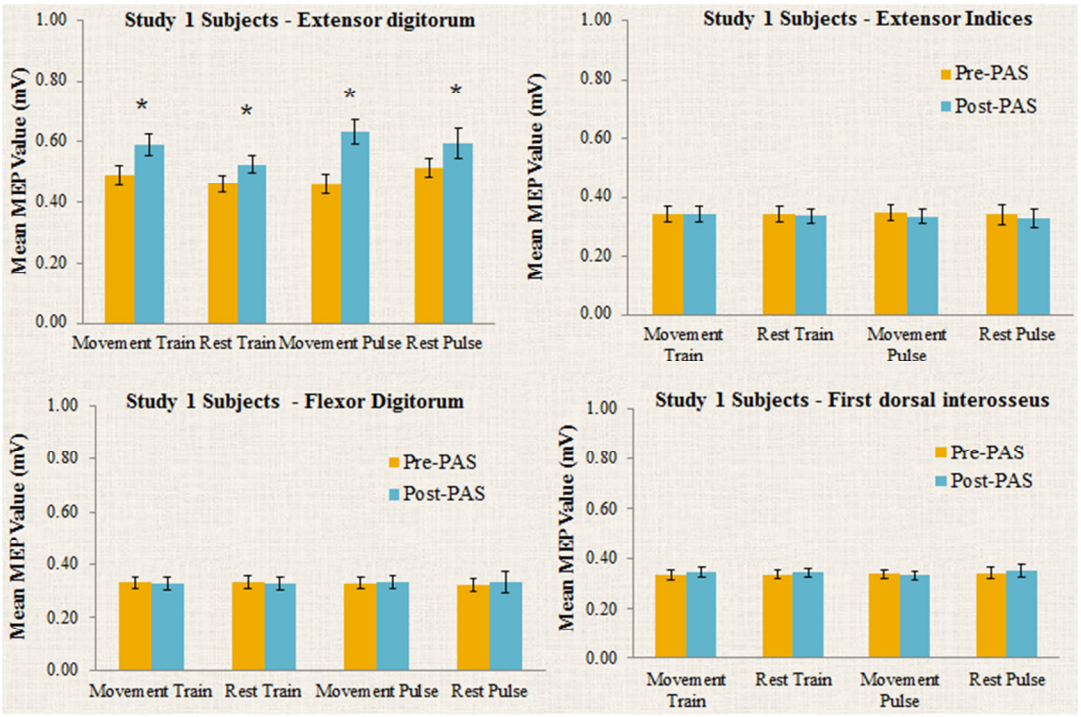

Figure 2.5 Only the target muscle (ED) showed significant changes in MEP amplitude. Group t test results of non-target muscles MEP changes (pre vs. post) for all four PAS protocols. FDS, flexor digitorum superficialis; ED, extensor indices; FDI, first dorsal interosseous; ADM, abductor digiti minimi; MEP, Motor evoked potential.

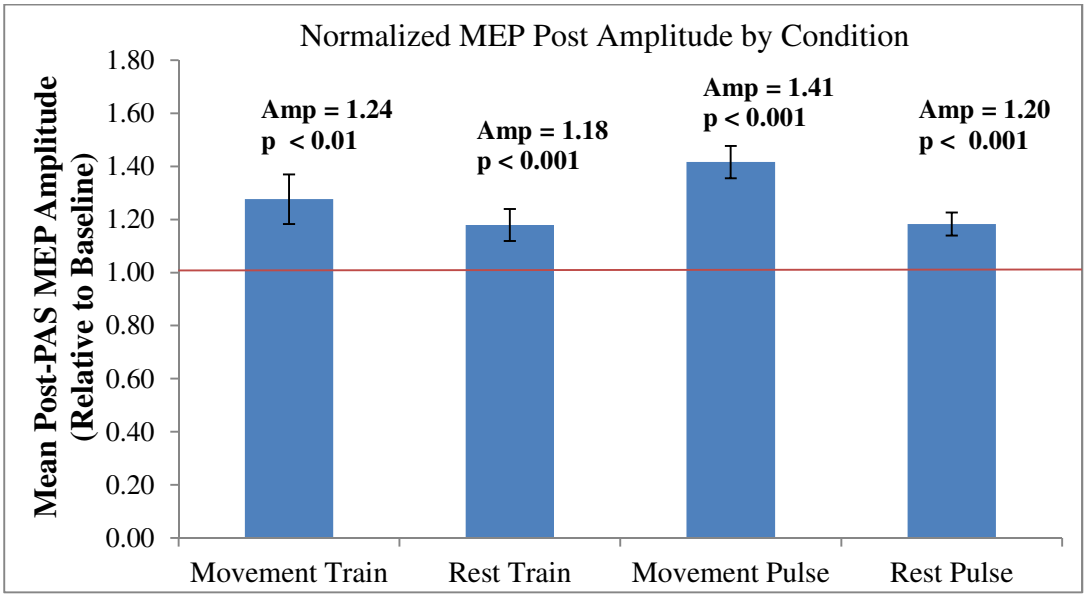

Figure 2.6 MEP amplitude post-PAS. Changes in MEP amplitude after ED targeted PAS. Mean (±SEM) MEP amplitude across subjects at rest, for each PAS protocol type (normalized to pre-PAS amplitude). MEPs were significantly increased for 30 min following PAS intervention, before returning to baseline by 90 min.

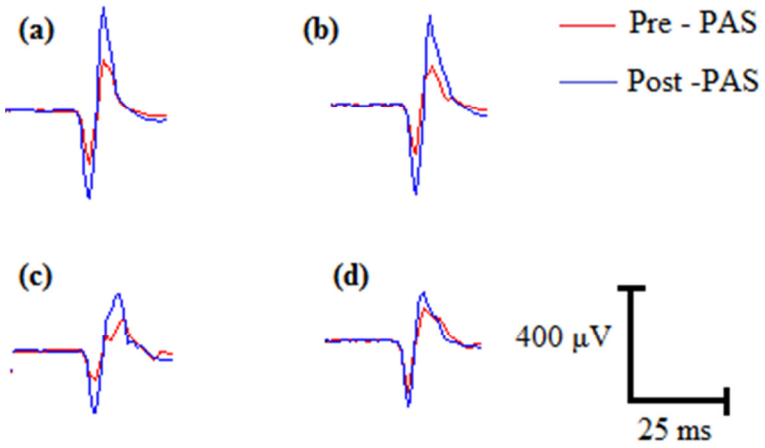

Figure 2.7 Averaged MEP waveform from one subject at rest pre and post PAS intervention. (a) Movement Pulse intervention, (b) Movement Train intervention (c) Rest Pulse intervention (d) Rest Train intervention. Experimental sessions occurred on separate days. These results reveal that 250 pairs of stimuli are sufficient to raise MEP amplitude, and when stimuli were timed to coincide with active finger extension, a greater increase in MEP amplitude was observed.

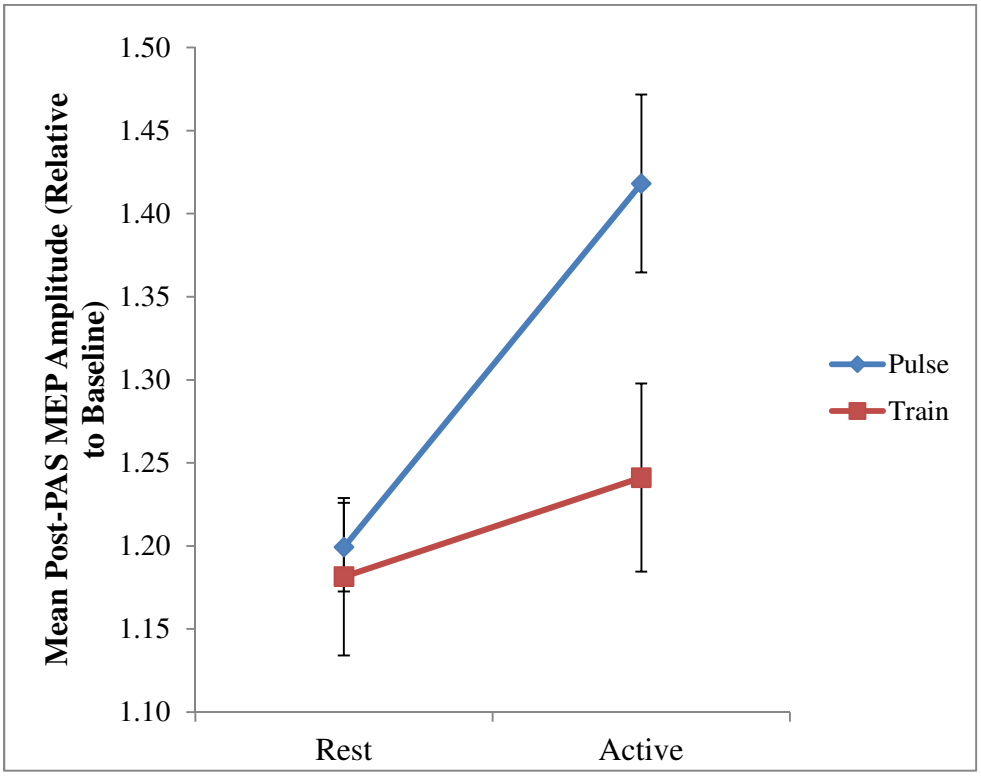

Figure 2.8 2x2 repeated measures ANOVA was performed to determine if stimulation type (pulse vs. train) during PAS intervention had an impact on MEP amplitude. The results show that behavior does have a significant ($p < 0.05$) effect on PAS-LTP like effects. This suggests that pulse protocols should be used for interventions with stroke subjects to maximize rehabilitation potential related to plasticity and excitability changes.

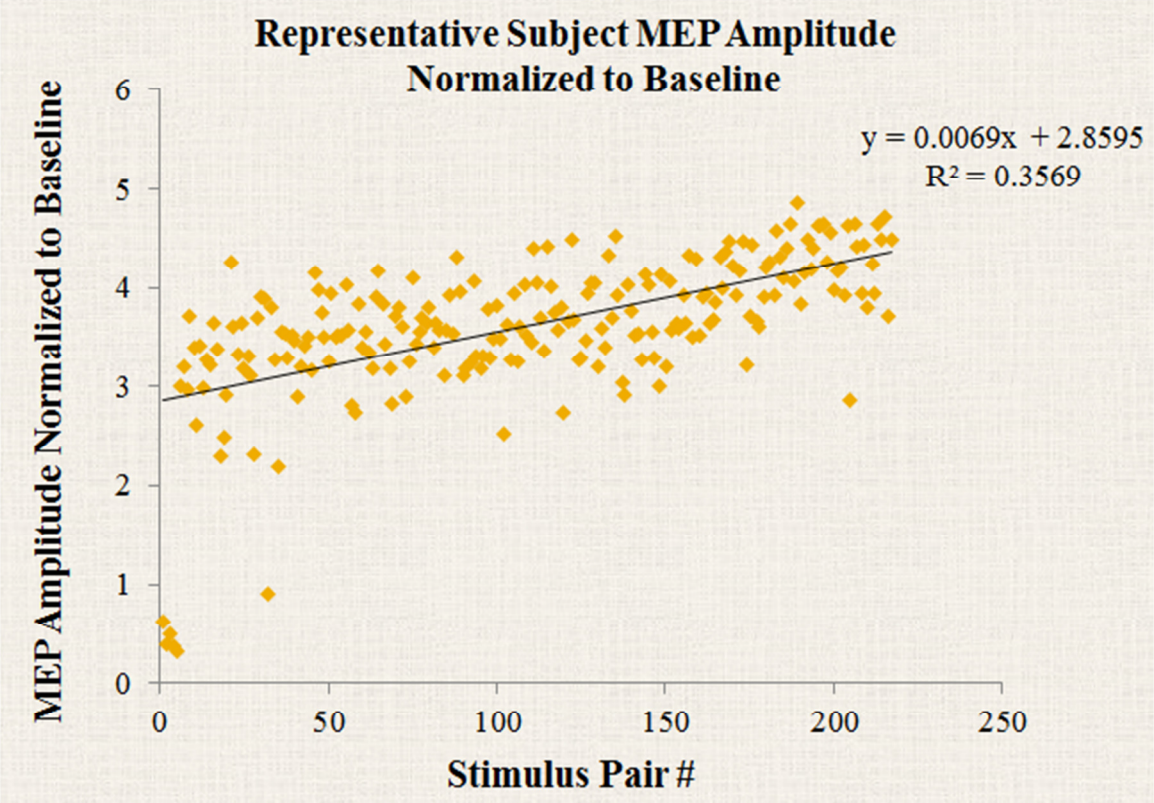

Figure 2.9 Motor-evoked potential amplitude during intervention (normalized to baseline amplitude). A clear, linear increase is seen throughout the stimulation session which leads to excitatory effects measured post-intervention.

2.4 Discussion

The present study provides evidence for the functional interaction of the repetitive coupling of active limb movement with PAS, which progressively increased human corticomotor excitability and in the ED and was sustained for up to 30 minutes following the intervention. These results show for the first time the potential optimal parameters to use when combining naturally occurring afferents generated by the actively moving limb in conjunction with PAS in an associative manner, such that when performed repetitively, has a short-term excitatory effect. In order to favor an excitability increase, we

recommend both active movements and single-pulse electrical stimulation. These parameters will be tested further in our next study.

In this regard, a positive effect of active movement was demonstrated across all subjects and is stronger if delivered with single-pulse stimulation frequency – i.e., 0.14 Hz shown in the subjects. The data suggest that single pulse electrical stimulation protocols should be used for interventions with stroke subjects to maximize rehabilitation potential related to plasticity and excitability changes. The current consensus on the mechanism of PAS-LTP like effects stresses the glutamatergic system, voltage-gated ion channels and the GABAergic system as "drivers" of neuroplastic adaptation. At glutamatergic synapses in the CNS binding to AMPA receptors of glutamate released by presynaptic activation, and the resulting postsynaptic depolarization which leads to removal of the Mg2+ block, together permit the influx of Ca2+ though the NMDA receptors [244, 286]. The magnitude and time course of the calcium flux will determine whether LTP or LTD is induced [88]. Transient, high calcium-fluxes invoke LTP, whereas sustained moderate calcium fluxes generate LTD, and low calcium fluxes do not induce adaptation [78, 104].

Studies have also shown that transient, high calcium-fluxes. As a result, the lower excitatory effect seen in both train stimulation conditions is possibly due to a more sustained calcium flux which depresses the excitatory effect of the intervention.

Movement caused an immediate increase in MEP amplitude, with a further progressive and significant increase when repeated for 250 cycles. This led to an effect of elevated MEP amplitude that persisted several minutes following the intervention period. Studies suggest the immediate PAS effect is limited to 90 minutes. Investigation into

determining the exact duration of the effects of movement single pulse PAS would further clarify the optimum time for PAS delivery as related to motor rehabilitation.

In this study, we compared the effects of different arrangements of paired stimulation, with the effects of the same low frequency (1Hz) stimulation alone by delivering 250 stimuli at 110% of the RMT at rest and found an increase in the MEP size following all interventions. Previous studies of 0.14 Hz stimulation over human primary motor cortex at rest show a short-term increase in corticomotor excitability (Chen, Seitz, 2002; Maeda, Pascal Leone et al., 2001). However, this is the first time the PAS-LTP like effect has been tested in this manner in the ED. The effect appears to be related to behavior as well as the number of applied electrical stimuli. In humans, long-term potentiation as few as 50 pulses may be required yet the effects can be quite variable across subjects [38]. We specifically used 0.14 Hz PAS but with sufficient repetitions to exert corticomotor excitability increases even in the absence of movement. We showed that stimulation alone with 250 repetitions was also able to increase MEP amplitude, but active movements showed a much more significant and larger increase. Of note, during the PAS intervention we observed variance in the onset and trajectory of MEP amplitude increase between individuals.

While both sub- and supra-threshold stimulation produce changes in the MEP, the effects of supra-threshold stimulation as performed in this study tend to exhibit a more reliable and robust pattern with prolonged number of stimuli [170, 174, 192]. In view of the current literature then, our findings of significant increase in MEP amplitude with the short intervention duration (30 min) and 110% RMT stimulus intensity are reassuring.

Upper limb movement forms a large basis for motor rehabilitation, and repetitive active movement can lead to a temporary reduction in spasticity and orthopedic benefit [151]. While the mechanism is incompletely understood, it is thought to result from effects of muscle spindle afferents at the spinal and cortical level [259]. The strong effects of movement-related afferents paired with TMS are significant and can be as long lasting as the effects of PAS alone and may persist for up to 60 minutes. This suggests that passive movement alone may be not result in any sustained change in excitability, which would be consistent with our results. The implication for the findings of the present study is that active movement during the muscle lengthening phase of movement might have therapeutic application in disorders of the upper limb as a result of stroke. However, more broadly, these findings suggest that the ability of PAS protocols to modulate cortical excitability may be influenced by interventions (such as movement and stimulus type) aimed at controlling cortical excitability.

Peripheral afferents lead to a cumulative and lasting effect that could occur at spinal and/or supraspinal levels. Furthermore, the excitatory phase of cyclic active movement may be complementary to an excitatory PAS protocol, and it may enhance the excitatory effect. For example, we have shown that that decreased afferent activity (rest PAS, associated with reduced MEP amplitude increase) also appears to decrease the efficacy of low frequency PAS. Moreover, the mechanism of our observed effect cannot be elucidated from the current protocol, yet PAS and active movement have separately been shown to alter both spinal [170] and cortical excitability [259]. The circumstances under which cortical and/or spinal excitability changes occur are influenced by the nature of the neuromodulatory protocol, where paired associative stimulation for example, can

change cortical but not spinal excitability [251]. In the present study, both spinal and cortical excitability changes could contribute to our findings; however, this remains to be determined.

Our findings support the idea that a movement and pulse type of associative paradigm could best be used to increase cortical excitability in the extrinsic ED muscle of the hand similar to the PAS effects seen in the intrinsic muscles. We have shown experimentally that the association of the facilitatory phase of movement with PAS repeatedly increase cortical excitability over time consistent with long-term potentiation, as currently is well demonstrated with PAS, yet this remains to be proven experimentally[227, 251]. We have shown that natural physiological activation of M1 (via voluntary movement) during the task synchronized with PAS results in a higher magnitude of associative LTP-like plasticity. This supports the claim that associative stimulation is a general principle for human neural plasticity. There are two forms of synaptic plasticity, which are homo and heterosynaptic plasticity. The homosynaptic plasticity refers to changes in the strength of a synapse due to its own activity, however, the heterosynaptic plasticity, is a change in the strength of a synapse due to activity in another pathway [160]. In our movement behavior PAS study, the induced M1 plasticity may be related to homosynaptic form of LTP/LTD as the change in MEP amplitude occurred largely in muscles innervated by the stimulated peripheral nerve in rest PAS, and a greater quantity of MEPs were found all the moving muscles after movement PAS.

The results of this study support the concept of topographical specificity possible with certain PAS interventions. The non-target muscle to exhibit the greatest number of measurable MEPs during movement single pulse PAS was the extensor indices (EI),

which is innervated by the posterior interosseous nerve, a distal branch of the radial nerve that supplies the ED. However, the trend in EI MEP amplitude was only increasing in the rest train protocol, and the result was statistically insignificant in all instances. In addition, the non-target muscle that came closest to statistical significance was a decreasing trend in the flexor digitorum superificialis, which is innervated by a branch of the median nerve. These findings support the proposition that PAS-induced adaptation represents a form of plastic neuromodification that is synapse-specific [170]. This topographical specificity [161] suggests that changes in excitability brought about by PAS are restricted to the cortical representations of muscles innervated by electrical stimulation and is consistent with previous findings of effects limited to muscles which share a common innervation as the target nerve/muscle [251, 279, 280].

We found that PAS paired with voluntary movement can optimally induce change in the corticospinal excitability and motor behavior that outlasted the stimulation period. The characteristics of this change are similar to associative LTP in animal models; as it rapidly developed (within 30 min), sustained 10 minutes after intervention, showed associativity (ISI < 20 ms failed to achieve significant excitation in M1, movement augmented the effect), and was input-specific (as M1 excitability changes were only detectable in "the moving" rather than "the resting" muscles (APB vs. DSF, EI and FDI). Additionally, this form of induced plasticity was timing-dependent, as its direction was governed by the order of TMS and the onset of voluntary movement. It is possible that the ISI (20 ms) may be too short to ensure LTP-like effects in all subjects due to differences in innate latency and the length of some of our subjects. A further

investigation into a longer ISI (25 ms) can determine if the additional delay will further increase the excitation obtained by this PAS intervention.

CHAPTER 3

IMPACT OF MOVEMENT TIMING AND INTERSTIMULUS INTERVAL

3.1 Abstract

Our previous studies established the ideal motor behavior and stimulation type to enhance the evoked response to transcranial magnetic stimulation (TMS) after training of the hand. This effect also depends on the latency of the preceding peripheral nerve stimulation (PNS) pulse. For intrinsic muscles of the hands, these latencies translate into interstimulus intervals (ISI) of greater than 20 milliseconds. In addition, we have shown that somatosensory afferents from the actively moving limb can alter corticomotor excitability. The repeated association of PNS with TMS is known to modulate corticomotor excitability; however, it is unknown whether these effects will be effected by longer ISI values and triggering of the paired stimulation earlier in finger movement. Thirteen healthy subjects received three PAS protocols which varied in the triggering method of the paired stimulation (EMG vs Movement) and the delay between electrical stimulation and TMS (20 vs 25 ms) during active extension movement, with the intervention order randomly assigned. Our results show that EMG triggered PAS correlated with earlier stimulation and a larger increase in M1 excitability compared to movement triggered stimulation. We also found that increasing the ISI had no significant effect on corticomotor excitability measurements. Thus, the association of somatosensory afferents from the moving limb with PAS is phase dependent, and that triggering of the

stimulation using EMG measurements would be ideal for use in modulating corticomotor excitability in stroke subjects and should be utilized in future therapeutic interventions.

3.1.1 Objective

Paired Associative Stimulation (PAS) has achieved distinction as a potential rehabilitative intervention for the treatment of neurological injury and disease. PAS is a valuable tool with which to examine Hebbian principles of neural plasticity in humans. Hebb's postulate states that When an axon of cell *A* is near enough to excite cell *B* or repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that *A*'s efficiency, as one of the cells firing *B*, is increased. Through PAS, two signals (afferent and efferent) arrive simultaneously at M1 in order to facilitate this Hebbian mechanism. Increases in the cortical response after PAS support the idea that joint activity of the synaptic units leads to a strengthening of synaptic efficiency. Prototypically, a single electrical stimulus is directed to a peripheral nerve in advance of transcranial magnetic stimulation (TMS) delivered to the contralateral primary motor cortex (M1). Repeated pairing of the stimuli (i.e., association) over an extended period may increase or decrease the excitability of corticospinal projections from M1, in manner that depends on the interstimulus interval (ISI). It has been suggested that these effects represent a form of associative long-term potentiation (LTP) and depression (LTD) that bears resemblance to spike-timing dependent plasticity (STDP) as it has been elaborated in animal models. Paired associative stimulation (PAS) combining peripheral electrical stimulation and transcranial magnetic stimulation (TMS) induces long term plasticity like changes in the corticospinal

projection to hand muscles in normal subjects [251]. This procedure allows for the study of Hebbian-like mechanisms of synaptic plasticity in the human motor cortex. If a weak excitatory input (afferent peripheral electrical stimulation) triggered 20 ms prior to a TMS pulse given over the target muscle area of the contralateral motor cortex, repeatedly arrives at cortical level, then a single pulse TMS of the target muscle area evokes a larger motor evoked potential (MEP) than before PAS. PAS protocols utilizing direct muscle stimulation have also been successful in producing LTP-like effects such as changes in intracortical excitatory circuits [164, 199]. We studied finger extensor muscles because the motor recovery of distal upper limb in post-stroke patients is a significant challenge to rehabilitation and is of particular importance in recovery of autonomy lost after stroke. We anticipated that finger movements for stroke patients would be more limited compared to healthy subjects so we sought to develop a novel EMG driven PAS paradigm using our previous virtual reality paradigm. This would allow for consistent PAS triggering upon muscle recruitment by the subject, regardless of the extent of extension achieved in the motion.

The current literature indicates that the effects of excitatory PAS are not necessarily limited to the muscles innervated nerve receiving electrical stimulation [38]. There are reported instances in which changes in the excitability of corticomotor projections induced by classic PAS protocols have been more pronounced for muscles that are innervated by a different nerve [257]. In response to our finding that excitatory effects in the ulnar nerve innervated ADM and non-target muscles were no significantly altered, we have found evidence for what has been referred to as a “somatotopic gradient.” [163, 251] Our previous findings imply that alterations in excitability brought

about by PAS are restricted to the cortical representations of muscles innervated by the target nerve/muscle that was stimulated electrically [251]. In our previous studies and current literature, it was noted that an increase in corticospinal excitability is achieved if the relative timing (ISI) is adjusted such that TMS is applied prior to the time at which the electrical afferent stimulation is anticipated to reach M1, repeated pairings can lead to a reduction in corticospinal excitability [285]. This timing dependency underscores the need to establish the excitatory timing necessary for use in the extensor digitorum Once established, this can be used to design excitatory protocols for rehabilitative purposes. Given the importance of the extrinsic finger extensors to functional use of the hand, the establishment of exact timing dependency necessitates particular attention.

In our previous studies on extrinsic muscles and in studies where the targets are intrinsic hand muscles, the interval between the peripheral nerve stimulus and the TMS pulse to generate sustained increases in corticomotor excitability is most commonly fixed (across participants) at 20 ms [162, 144, 249, 252, 288, 300]. Recent literature has also demonstrated that an ISI of 25 ms may have similar effects [279, 280]. Our study sought to establish if these longer ISI values would also allow for excitability increases. We hypothesized that since stroke patients tend to have longer hand muscle latencies than healthy individuals, a longer ISI would be more favorable for stroke subjects. It is also worth noting that the effects of these protocols can vary significantly across participants [132]. In our previous studies on ten healthy subjects PAS protocols with ISIs < 20 ms, three subjects showed the expected increase in corticospinal excitability, whereas the other seven exhibited a decrease (mean ratio post-PAS/pre-PAS = 1.00; range = 0.36–

1.17). The longer delay was expected to increase the strength of the group excitatory response.

In previous forms of PAS, the peripheral electrical stimulation induces activity in M1 through corticocortical fibers from the somatosensory cortex [280]. However, it has been rarely investigated whether other types of afferent input to M1 combined with TMS techniques can produce similar associative LTP-like effects or not [81, 155]. Studies combining voluntary movement and TMS or movement and PAS have been performed on the lower extremities; others have used exercise prior to PAS in order to prime the excitatory effects on the upper limb [162, 245]. These studies have framed the PAS effect in humans as similar to spike-timing-dependent plasticity, due to the dependence on the polarity of the effect (excitatory, inhibitory) on the order of the stimuli. It remains the case however, that few empirical studies have combined PAS and voluntary movement to scrutinize the role of the timing mechanism between finger movement and stimulation in the facilitation of excitability.

In the present study, our scope was twofold. First we investigated the effect of PAS application during voluntary muscle contraction on motor cortical excitability with two different ISI values. Second we examine the effect of stimulus timing relative to EMG activity on excitability. We sought to test directly whether the ISI and movement-timing of stimulation affects corticomotor excitability, by studying MEP amplitude changes after PAS training. We measured MEPs with the assumption that any change in the corticospinal excitability following PAS represent and excitability change at the level of the primary motor cortex. We investigated the effect of one session of paired associative stimulation on the excitability of the corticospinal projection to extensor

digitorum (ED) muscle (MEP amplitude before and after PAS) in healthy subjects. We sought to establish the effects of interstimulus interval (20 ms vs. 25 ms) and the respective role of the timing of peripheral electrical stimulation during finger movement ts (EMG vs. Kinematics) in facilitating motor excitability. Our published and preliminary data suggest that our virtual reality (VR) environments provoke a sense of reality to the subjects, provide valid and reliable measures of hand kinematics, and possess potential as a rehabilitation tool in clinical populations [6, 7, 17]. Our preliminary data also established that interstimulus intervals lower than 20 ms were unable to affect significant increases in M1 excitability so 20 ms was chosen as the ISI across all four protocols. It will be shown that our PAS protocol was able to induce significant changes in excitability of the extensor digitorum motor cortex area in healthy subjects and that an EMG triggered, movement-single pulse design with an ISI of 25 ms will be optimal for use in the rehabilitation of the hand post-stroke.

3.2 Methods

3.2.1 Subjects

Thirteen right-handed healthy individuals (5 male, 3 female; age 23-30 years) volunteered after giving their written informed consent to the study approved by the Rutgers and NJIT institutional review boards. Subjects attended on three separate occasions in a within-subjects study design. Each visit the subjected participated in one of three possible PAS interventions: (1) Movement triggered single pulse PAS_{20} (2) EMG

triggered single pulse PAS_{20} (3) Movement triggered single pulse PAS_{25}. Each PAS protocol was randomly assigned to the visit.

3.2.2 Procedure

The subject was seated with their right arm supported at the hand, wrist and elbow in a custom built armrest. The wrist was slightly flexed and the apparatus allowed for 90º of finger extension/flexion. The forearm was positioned partially supine on a curved, padded rest so that the ulnar styloid sat just on the edge of the padding. Their arm was positioned such that the subject's arms were aligned with a pair of onscreen virtual hands created using Virtools Software (Dassault Systems) on a TV display. The fingers were placed in a relaxed, flexed position at the MCP, PIP and DIP joints. To maximize the perception of realness, the TV was positioned horizontally above the hands and angled so that the vantage point of the virtual hands, which was driven by glove data, corresponded to the subject's actual hands underneath the TV (Figure 3.1). Our published data suggest that this setup provides a sense of ownership of the virtual hands and we have successfully employed the virtual reality hand feedback in a number of healthy and patient-based studies.

3.2.3 Movement-triggered Single Pulse

Initial hand position was recorded by a Cyberglove (CyberGlove Systems) wired 22 sensor data glove. All hand movements were recorded by the glove which was calibrated for each subject (VirtualHand Software, CyberGlove Systems). Once calibrated, the subject's hand movements were shown in real time on the display. Each subject was

asked to follow the onscreen commands. Text commands instructing the subjects to open their hands were displayed in the virtual above the hands environment. Each time the OPEN command was displayed, the subject would extend their fingers and a MCP joint extension of 25º from the resting position triggered the delivery of the paired stimulation (e-stim and TMS were separated by 20 milliseconds). Once their finger reached the displayed target, they were to return to the rest position. PAS_{20} was applied every 4-6 seconds (duration was random) and continued for 30 minutes for a total of 250 paired stimulations.

3.2.4 EMG-triggered Single Pulse

Subject position and the glove calibration was identical to the movement triggered PAS_{20} but now the paired stimulation was triggered by EMG activity. Maximum voluntary contraction of the ED was recorded using EMG bipolar electrodes and used as a baseline measurement. The paired stimulation paradigm combined TMS and peripheral electrical stimulation of the extensor digitorum. The peripheral stimulation was a single 1-millisecond shock triggered by EMG activity of the ED described in Chapter 3 which repeated every 4-6 seconds over the belly of the extensor digitorum through surface electrodes. TMS was delayed by 20 milliseconds with respect to the onset of the electrical pulse. Both TMS and electrical stimulation were applied at an intensity that evoked a just-visible motor response in the extensor digitorum. A custom built MATLAB program recorded EMG activity during the training and paired stimulation was triggered by EMG activity equal to between 15-30% of maximum voluntary contraction in the ED that was maintained for at least 100 ms. Duration of PAS, interval between trials and total

stimuli delivered was the same as movement triggered PAS_{20} (30 minutes for a total of 250 paired stimulations).

For Movement triggered single pulse PAS_{25} the setup was identical to the conditions used in Movement triggered single pulse PAS_{20} save for the ISI value was changed to 25 milliseconds. PAS_{25} was applied every 4-6 seconds (duration was random) and continued for 30 minutes for a total of 250 paired stimulations.

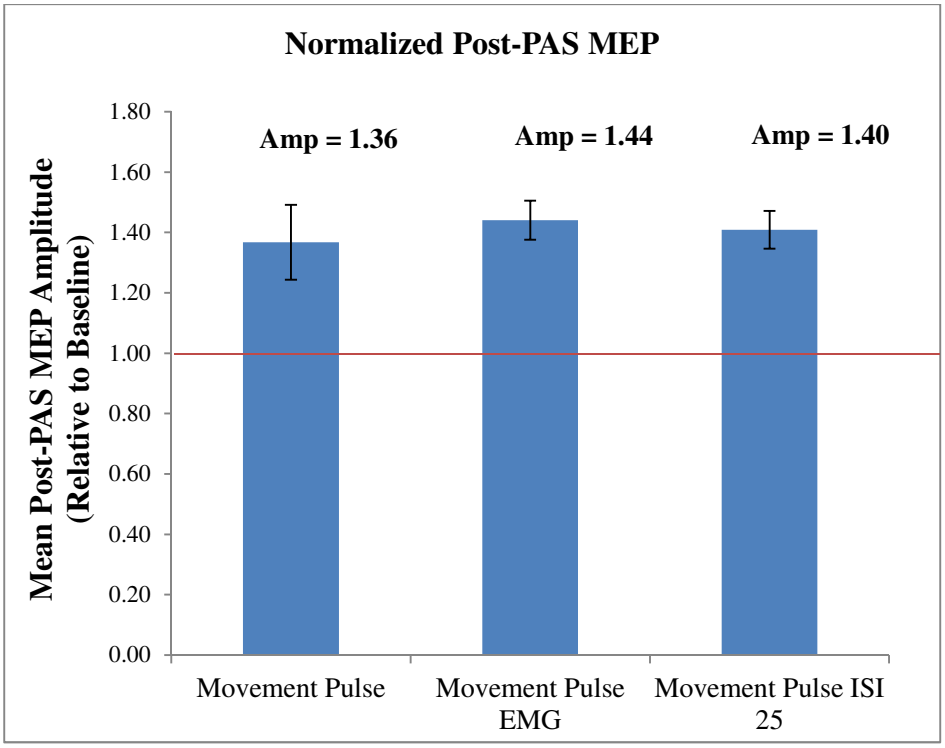

Figure 3.1 Comparisons of the excitatory effects of the three PAS protocols from Study 3. All three protocols induced significant increases in MEP amplitude relative to baseline (AMP > 1) while rmANOVA analysis revealed no significant effect of trigger type or ISI on group effect.

Surface electromyographic (EMG) activity was recorded from five wireless Trigno™ electrodes (Delsys Inc.) placed over the muscle belly of the right extensor digitorum, right flexor digitorum, right flexor digitorum indicis, right extensor indicis and

the abductor digit minimi. EMG signals were amplified and band-pass filtered before being digitized at 1000 Hz. EMG was recorded continuously and then processed with each MEP comprising a window spanning 50 ms prior and 100 ms after stimulation using a custom built MATLAB acquisition and analysis system (Mathworks Inc.). To compare the timing of stimulation relative to muscle activity, a 1500 ms second window prior to electrical stimulation delivery was examined. An EMG envelope was created and the maximum muscle activity was calculated for the window. Next we calculated the time (ms) between the ED muscle reaching 10% of maximum EMG activity reached prior to e-stim and the delivery of e-stim. In the absence of kinematic data , this value was used to estimate how early stimulation was triggered during muscle activation.

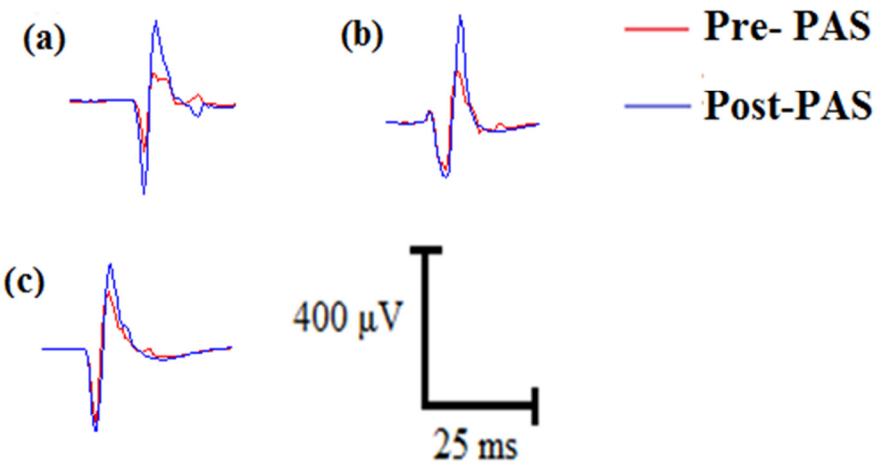

Figure 3.2 Averaged MEP waveform from one representative subject pre and post PAS intervention. (a) Movement single pulse intervention, (b) Movement ISI 25 single pulse intervention (c) Movement EMG triggered single pulse intervention (d) Rest train intervention. Experimental sessions occurred on separate days. These results reveal that 250 pairs of stimuli are sufficient to raise MEP amplitude, and when stimuli were timed to arrive earlier in finger movement, a greater increase in MEP amplitude was observed.

Single-pulse TMS (Magstim Rapid2, 70mm double AFC coil) was applied at 100% of the resting motor threshold, the minimum intensity required to elicit MEPs > 50 μV in the right extensor digitorum (ED) muscle in 4/6 consecutive trials. For subjects who had undergone MRI, a high-resolution anatomical MRI scan (3T Siemens Allegra) was used to render a 3-D cortical surface. A visor with motion tracing markers was placed on the subjects head. Fiducial locations on the MRI were core-registered with the subjects head to allow frameless neuronavigation (Visor, Advanced Neuro Technology). The optimal site of stimulation for ED (i.e., the hotspot), determined from initial exploration, was defined as the site with the largest MEPs for a given supra-threshold stimulus intensity, and used throughout the experiment. The stimulated ED hotspot of the motor cortex was marked on the MRI scan. The coil was held tangentially with the handle facing 45º posteriorly off the sagittal plane, and was tracked online to be stay over the ED hotspot. For subjects without an anatomical MRI, their head was co-registered to a model MRI.

During PAS_{20}, TMS was triggered 20 ms after peripheral electrical stimulation was delivered to ensure both signals arrived at the cortex simultaneously. For PAS_{25} the delay was set for 25 ms. In all cases, 250 paired pulses were delivered to the ED hotspot at 110% RMT with a frequency of approximately 0.14 Hz. The level of attention, a significant modulator of PAS-LTP effects [252], was controlled and attention was maximized to the simulated hand by a color change on the virtual display when the command changed. For all three conditions, the subjects were asked to count and report the total number of stimuli they received as correctly as they could at the end of PAS.

The interventional paired stimulation was performed with electrical stimulation of the target muscle (innervated by the radial nerve) by placing the bipolar electrodes just proximal to the muscle belly of the ED. Intensity was chosen as 110% of that sufficient to produce a just noticeable twitch in the ED muscle at rest (8.3 ± 1.7 mA, 300 V_{max}, n =8). Stimulus was performed with a Digitimer DS7A stimulator (Digitimer Ltd.) using constant current square wave pulses (cathode proximal, stimulation width 1 ms) followed 20ms later by TMS. Single pulse PAS consisted of 250 stimulus pairs at a frequency of 0.2 Hz. Train PAS consisted of trains of 500 ms duration consisting of 1 ms square waves delivered at 10 Hz (i.e., 5 stimuli per train) with TMS delivered 20ms after the last shock of the train.

Each subject was randomly assigned to a PAS treatment order (Figure 3.2). Time between sessions varied from 1-12 days. Forty MEPs (using 110% RMT) were collected pre and post intervention over the course of 4 minutes. To maintain consistent EMG activity across trials and conditions, subjects were asked to relax their hands (monitored by real-time EMG) in the neutral start position when not opening or closing their hand. The time between treatments on the same day was a minimum of 60 minutes to allow for sufficient washout of any previous PAS effects [252].

3.2.5 Statistics

Electrophysiological variables (MEP, EMG) were averaged across trials for each condition and subject. Means were submitted to a repeated-measures analysis of variance (rmANOVA). Four paired t-tests were used to compare pre and post PAS MEP amplitudes across all three protocols (using a Bonferonni correction). Finally,

rmANOVA was also performed for to characterize the degree to which each condition (ISI, trigger type) contributed to the MEP excitatory effect. For this, MEP was defined as the dependent variable stimulation type and behavior (movement, rest) as independent variables. Data were analyzed with PASW Statistics 18 (SPSS). rmANOVA was used to test for main effects and interactions. Statistically significant interaction effects were tested post hoc by Tukey's honestly significant difference (HSD) test. Significance threshold was set at $P < 0.05$.

3.3 Results

3.3.1 Measurements of Cortical Excitability

The peak-to-peak amplitude for means MEPs increased significantly for each MEP was measured. The post-PAS data was grouped by intervention type (movement and single pulse; rest and single pulse; movement and train; rest and train) and referred to as Movement single pulse, Movement train, Rest single pulse and Rest train, respectively. The mean MEP amplitude for each group was expressed as a ratio to mean pre-intervention amplitude. Group mean data post-PAS was tested for normality, and then compared to pre-PAS baseline using a 2x2 ANOVA.

To test the immediate effect of active movement on MEP amplitude relative to the resting protocol, the mean of approximately 40 post MEPs was examined with respect to pre-intervention using repeated measures ANOVA, Data are presented as mean ± SEM. 'Normalized' data refers to expression as a ratio to pre-intervention baseline. The results show that behavior does have a significant ($p < 0.05$) effect on PAS-LTP like

effects. This suggests that active protocols should be used for interventions with stroke subjects to maximize rehabilitation potential related to plasticity and excitability changes.

A statistically significant increase in the MEP amplitude was observed in the group (n = 13) in both PAS_{20} and PAS_{25} (Figure 3.3). While the PAS_{25} increases in M1 excitability were slightly smaller than the effects seen in the $PAS_{20,}$ paired t-tests revealed no significant difference between the changes. Repeated measures ANOVA revealed no significant interaction for ISI on the excitatory effect observed in the ED. Data showing representative sample resting MEP waveforms for one subject at baseline and post-intervention for PAS_{20}, PAS_{25} and PAS_{EMG} reveals post PAS intervention MEP amplitude (mV) was significantly increased in all three conditions: PAS_{20} = 0.56 ± 0.17 (142 ± 18%, $P < 0.05$); PAS_{25} = 0.53 ± 0.19 (144 ± 16%, $P < 0.05$). MEP amplitudes measured after PAS_{20} were lower than those taken after PAS_{25} however, paired t-tests (Figure 3.4) and rmANOVA analysis demonstrates that the interstimulus interval did not have a significant effect on excitation ($F(1,12) = 8.7$, $p = 0.12$).

Both PAS_{EMG} and PAS_{20} demonstrated significant increases M1 excitability as measured by MEP amplitude. PAS_{EMG} and PAS_{20} facilitated an increase in MEP amplitude (n = 13; mean ± SEM): PAS_{EMG} = 0.53 ± 0.15 (144±16 %), $p < 0.001$; PAS_{20} = 0.56 ± 0.18 (142±18 %), $p < 0.001$). Analysis of the trigger timing was performed as described in the methods section (n = 10). The results showed that the EMG triggered protocol resulted in an average stimulation that arrived 14 milliseconds prior to the average extension triggered stimulation (PAS_{20} = 419 ± 18; PAS_{EMG} = 393 ± 6; $p < 0.01$). There were fewer missed stimulations using the EMG triggered protocol (0.8% vs. 2.2%) however, this difference failed to reach significance (n =10; $p = 0.23$). This decrease in

time between target EMG activity and stimulation was reflected in a greater increase in excitability as measured by MEP amplitude (Figure 3.5). Paired t-tests revealed this difference was not significant. A repeated measures ANOVA determined that the relative timing of each stimulation used during PAS had no significant impact on the excitatory effect on MEP amplitude ($F(1,12) = 11.24$, $p = 0.41$). The results show that single pulse stimulus has the greater excitatory effect on M1 compared to the train stimulation.

3.4 Discussion

Our previous studies demonstrated that induced human associative plasticity in the motor cortex is by combining movement, peripheral electrical nerve stimulation and TMS. Also, we found that associative stimulation of motor cortices can induce lasting excitability changes in the target M1. These results have also underscored the importance of the temporal order of presynaptic and postsynaptic spiking of associative stimulation, and have defined temporal windows of tens of milliseconds for the polarity of induced plasticity which is supported by the PAS literature for the upper limb [37, 47, 53, 60]. Since PAS plasticity effects are believed to arise from a form of spike timing-dependent plasticity (STDP) it was important to define the postsynaptic spiking time window as well as possible. This is especially important when seeking a specific effect because spiking the target cortical with TMS after peripheral stimulation leads to LTP, while TMS spiking before synaptic activation leads to LTD [176].

We found significant increase in MEP amplitude of the right ED muscle for PAS_{20}, PAS_{25} and PAS_{EMG}. However, this change in excitability was not generalized to the non-target muscles, and no MEPs were elicited in the ADM, which was not involved

in the voluntary movement task. This finding suggests that M1 plasticity in PAS is governed by strict somatotopy based on muscle activity in movement task. This somatotopy is consistent with other associative plasticity protocols [56, 88, 110-113]. In human studies, the motor potential (MP) component of movement related cortical potential (MRCP) occurs partly before and after the EMG onset. This potential probably represents activation of pyramidal tract neurons in M1, and persists for 30 –50 ms after the onset of EMG activity [202, 211].

Animal studies have shown that M1 directly and indirectly receives multiple inputs from other cortical and subcortical regions that may play important roles in motor processing, including premotor, supplementary motor, cingulated motor, somatosensory and prefrontal cortex, and anterior thalamic nuclei which indirectly connect cerebellum and basal ganglia outputs to M1[22, 66, 82, 84, 90]. During movement execution, top-down synchronous firing of pyramidal neurons occurs in all layers (including layers 2/3 and 5) of M1 [133]. The activity of those neurons stops at the end of movement execution and replaced by activation of other neuronal subsets in layers 4 and 6 during movement-off and post-movement phases of motor action [133, 159].

In conclusion, this study provides a new associative stimulation TMS protocol that can be used for induction of M1 plasticity in chronic stroke subjects. In previous protocols, peripheral electrical stimulation and contralateral M1 conditioning TMS were used to induce M1 plasticity [53-56, 150, 152]. However, in our movement PAS protocols, intrinsic M1 activation was also used. Our study may provide the evidence that associative stimulation-induced plasticity is a rather principle dependent on the nature of used stimuli. The finding that PAS may induce M1 plasticity supports the possibility of

its use for rehabilitation of neurological disability after vascular, inflammatory or degenerative brain diseases [44, 67-70, 81, 155,162,190]. Since topographical specificity is an important characteristic of MRCS-induced plasticity, it can be used to induce movement-specific M1 plasticity, rather than generalized increase/decrease of M1 plasticity induced by rTMS [33, 41, 86] or tDCS [59, 77, 221], which can be tailored to match different rehabilitation situations.

The present study provides evidence for the functional interaction of the repetitive coupling of active limb movement with PAS, which progressively increased human corticomotor excitability and in the ED and was sustained for up to 30 minutes following the intervention. These results show for the first time the potential optimal parameters to use when combining naturally occurring afferents generated by the actively moving limb in conjunction with PAS in an associative manner, such that when performed repetitively, has a short-term excitatory effect. In order to favor an excitability increase, we recommend both active movements and single-pulse electrical stimulation. These parameters will be tested further in our next study.

In this regard, a positive effect of active movement was demonstrated across all subjects and is stronger if delivered with single-pulse stimulation frequency – i.e., 0.14 Hz shown in the subjects. The data suggest that single pulse electrical stimulation protocols should be used for interventions with stroke subjects to maximize rehabilitation potential related to plasticity and excitability changes. The current consensus on the mechanism of PAS-LTP like effects stresses the glutamatergic system, voltage-gated ion channels and the GABAergic system as “drivers” of neuroplastic adaptation. At glutamatergic synapses in the CNS binding to AMPA receptors of glutamate released by

presynaptic activation, and the resulting postsynaptic depolarization which leads to removal of the Mg^{2+} block, together permit the influx of Ca^{2+} though the NMDA receptors [122, 164]. The magnitude and time course of the calcium flux will determine whether LTP or LTD is induced [207]. Transient, high calcium-fluxes invoke LTP, whereas sustained moderate calcium fluxes generate LTD, and low calcium fluxes do not induce adaptation. As a result, the lower excitatory effect seen in both train stimulation conditions is possibly due to a more sustained calcium flux which depresses the excitatory effect of the intervention.

Movement caused an immediate increase in MEP amplitude, with a further progressive and significant increase when repeated for 250 cycles. This led to an effect of elevated MEP amplitude that persisted several minutes following the intervention period. Studies suggest the immediate PAS effect is limited to 90 minutes [56, 61, 90]. Investigation into determining the exact duration of the effects of movement single pulse PAS would further clarify the optimum time for PAS delivery as related to motor rehabilitation.

In this study we compared the effects of different arrangements of paired stimulation, with the effects of the same low frequency (0.141Hz) stimulation alone by delivering 250 stimuli at 110% of the RMT at rest and found an increase in the MEP size following all interventions. Previous studies of 0.14 Hz stimulation over human primary motor cortex at rest show a short-term increase in corticomotor excitability [28, 32]. However, this is the first time the PAS-LTP like effect has been tested in this manner in the ED. The effect appears to be related to behavior as well as the number of applied electrical stimuli. In humans long-term potentiation as few as 50 pulses may be required

yet the effects can be quite variable across subjects [38]. We specifically used 0.14 Hz PAS but with sufficient repetitions to exert corticomotor excitability increases even in the absence of movement. We showed that stimulation alone with 250 repetitions was also able to increase MEP amplitude, but active movements showed a much more significant and larger increase. Of note during the PAS intervention was the varied onset and trajectory of MEP amplitude increase between individuals.

While both sub- and supra-threshold stimulation produce changes in the MEP, the effects of supra-threshold stimulation as performed in this study tend to exhibit a more reliable and robust pattern with prolonged number of stimuli [170, 174, 192]. In view of the current literature then, our findings of significant increase in MEP amplitude with the short intervention duration (30 min) and 110% RMT stimulus intensity are reassuring.

Upper limb movement forms a large basis for motor rehabilitation, and repetitive active movement can lead to a temporary reduction in spasticity and orthopedic benefit [151]. While the mechanism is incompletely understood, it is thought to result from effects of muscle spindle afferents at the spinal and cortical level [259]. The strong effects of movement-related afferents paired with TMS are significant and can be as long lasting as the effects of PAS alone and may persist for up to 60 minutes. This suggests that passive movement alone may be not result in any sustained change in excitability, which would be consistent with our results. The implication for the findings of the present study is that active movement during the muscle lengthening phase of movement might have therapeutic application in disorders of the upper limb as a result of stroke. However, more broadly, these findings suggest that the ability of PAS protocols to

modulate cortical excitability may be influenced by interventions (such as movement and stimulus type) aimed at controlling cortical excitability.

Peripheral afferents lead to a cumulative and lasting effect that could occur at spinal and/or supraspinal levels. Furthermore, the excitatory phase of cyclic active movement may be complementary to an excitatory PAS protocol, and it may enhance the excitatory effect. For example, we have shown that that decreased afferent activity (rest PAS, associated with reduced MEP amplitude increase) also appears to decrease the efficacy of low frequency PAS. Moreover, the mechanism of our observed effect cannot be elucidated from the current protocol, yet PAS and active movement have separately been shown to alter both spinal [170] and cortical excitability [259]. The circumstances under which cortical and/or spinal excitability changes occur are influenced by the nature of the neuromodulatory protocol, where paired associative stimulation for example can change cortical but not spinal excitability [251]. In the present study, both spinal and cortical excitability changes could contribute to our findings; however, this remains to be determined.

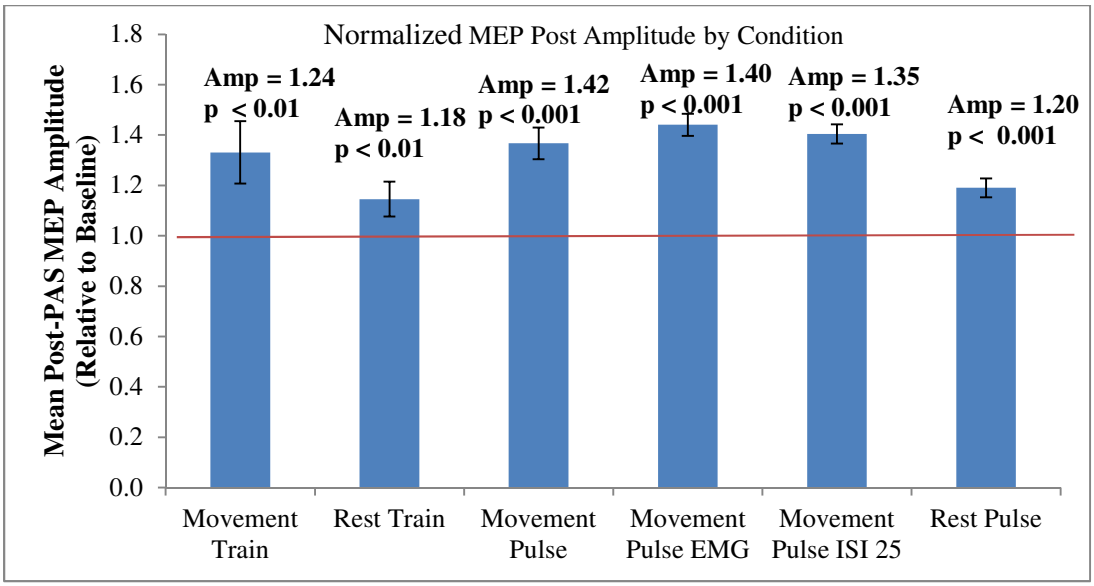

Figure 3.3 Group data for the thirteen subjects who completed all six PAS protocols along with Study 1 results for the participants; t tests show significant excitation for all groups in all six protocols, with the Movement Pulse inducing the largest changes.

Our findings support the idea that a movement and pulse type of associative paradigm could best be used to increase cortical excitability in the extrinsic ED muscle of the hand similar to the PAS effects seen in the intrinsic muscles. We have shown experimentally that the association of the facilitatory phase of movement with PAS repeatedly increase cortical excitability over time consistent with long-term potentiation, as currently is well demonstrated with PAS, yet this remains to be proven experimentally [227, 251]. We have shown that natural physiological activation of M1 (via voluntary movement) during the task synchronized with PAS results in a higher magnitude of associative LTP-like plasticity. This supports the claim that associative stimulation is a general principle for human neural plasticity. There are two forms of synaptic plasticity, which are homo and heterosynaptic plasticity. The homosynaptic plasticity refers to changes in the strength of a synapse due to its own activity, however, the heterosynaptic plasticity, is a change in the strength of a synapse due to activity in another pathway

[160]. In our movement behavior PAS study, the induced M1 plasticity may be related to homosynaptic form of LTP/LTD as the change in MEP amplitude occurred largely in muscles innervated by the stimulated peripheral nerve in rest PAS, and a greater quantity of MEPs were found all the moving muscles after movement PAS.

The results of this study support the concept of topographical specificity possible with certain PAS interventions. The non-target muscle to exhibit the greatest number of measurable MEPs during movement single pulse PAS was the extensor indices (EI), which is innervated by the posterior interosseous nerve, a distal branch of the radial nerve that supplies the ED. However, the trend in EI MEP amplitude was only increasing in the rest train protocol and result was statistically insignificant in all instances. In addition, the non-target muscle that came closest to statistical significance was a decreasing trend in the flexor digitorum superificialis, which is innervated by a branch of the median nerve. These findings support the proposition that PAS-induced adaptation represents a form of plastic neuromodification that is synapse-specific [170]. This topographical specificity [161] suggests that changes in excitability brought about by PAS are restricted to the cortical representations of muscles innervated by electrical stimulation and is consistent with previous findings of effects limited to muscles which share a common innervation as the target nerve/muscle [251, 279, 280].

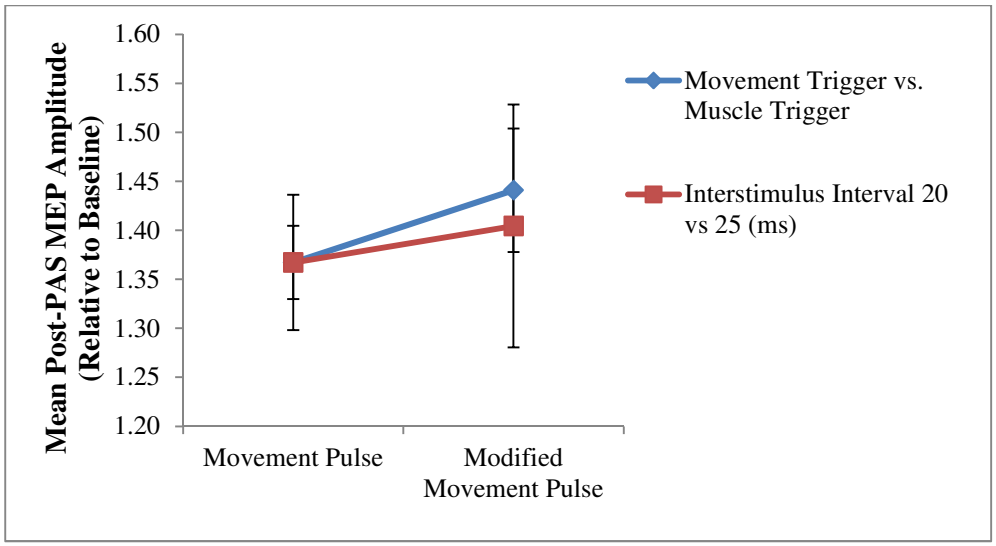

Figure 3.4 2x2 repeated measures ANOVA was performed to determine if ISI (20 vs. 25) or relative stimulation time during PAS intervention had an impact on MEP amplitude. The results show that neither relative timing ($F(1,12) = 8.44$, $p = 0.12$) nor ISI ($F(1,12) = 11.88$, $p = 0.41$) have a significant effect on the induction of PAS-LTP like effects.

We found that EMG triggered PAS paired with voluntary movement can optimally induce change in the corticospinal excitability and motor behavior that outlasted the stimulation period. The characteristics of this change are similar to associative LTP in animal models; as it rapidly developed (within 30 min), sustained at least 10 minutes after intervention, showed associativity (ISI < 20 ms failed to achieve significant excitation in M1, movement augmented the effect), and was input-specific (as M1 excitability changes were only detectable in "the moving" rather than "the resting" muscles (APB vs. DSF, EI and FDI). Additionally, this form of induced plasticity was timing-dependent, as its direction was governed by the order of TMS and the onset of voluntary movement. It is possible that the ISI (20 ms) may be too short to ensure LTP-like effects in all subjects due to differences in innate latency and the length of some of our subjects. A further investigation into a longer ISI (25 ms) can determine if the additional delay will further increase the excitation obtained by this PAS intervention.

The present study provides evidence for the functional interaction of the repetitive coupling of phase-specific passive limb movement with TMS over primary motor cortex, which progressively reduced human corticomotor excitability and was sustained for 20 minutes following the combined intervention, yet no such reduction was observed during or following intervention with the stimulation only. These results show for the first time that naturally occurring afference generated by the actively moving limb can be harnessed to interact with corticomotor activity from PAS in an associative manner, such that when performed repetitively, and has a short-term neuromodulatory effect. In order to favor an excitability reduction modulatory effect in the present study, we selected both electrical stimulation rate and ISI, accordingly. In this regard, an effect of movement was demonstrated that may be stronger if delivered at for longer duration of with multiple sessions and with a similar stimulation frequency – i.e., 0.2Hz shown in one subject and consistent with our previous work [17].

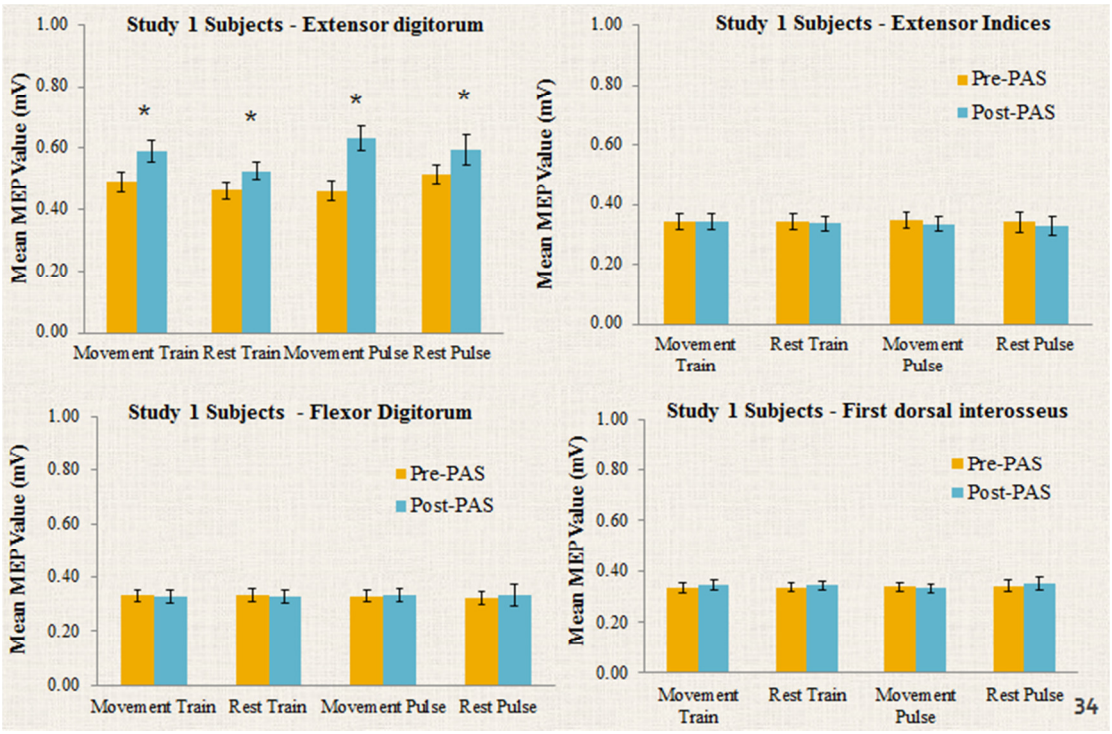

Figure 3.5 Group t test results of target and non-target muscles MEP changes (pre vs. post) for all four PAS protocols. FDS, flexor digitorum superficialis; ED, extensor indices; FDI, first dorsal interosseous; ADM, abductor digiti minimi; MEP, Motor evoked potential.

Movement caused an immediate increase in MEP amplitude, with a further progressive and significant increase when repeated for 250 pairs. This led to an effect of increased MEP amplitude and reduced RMT that persisted following the intervention period, and then returned to baseline within 30 minutes. Stimuli delivered at the end of range of muscle lengthening resulted in lower changes in MEP amplitude after the intervention, supporting previous literature that excitability changes are phase specific [5], and that movement alone at this rate does not have cumulative effects on MEP amplitude [17].

Previously, we used 0.4 Hz PAS but with insufficient repetitions to exert CM excitability increases even in the absence of movement. We showed that stimulation of

250 repetitions did increase MEP amplitude and a trend of increased excitability was present. Of note during the intervention, was the different onset and trajectory of MEP amplitude increase during the intervention between individuals. The variance in intervention effect time-course has been observed previously with other repetitive non-invasive stimulation protocols (rTMS, [24]; SAS, [11]). We suspect that while the dose of stimulation might be consistent across subjects for neuromodulation paradigms such as this, the individual response time-course will be different for a host of reasons, including brain state. Until real time individual dose–response is sufficiently considered, we might expect variance in the lasting excitability changes. This remains to be further explored in the Chapter 4 of this dissertation well as future neuromodulation protocols.

It is possible that the stimulus intensity we used results in a net facilitatory effect similar to paired pulse techniques that use a facilitatory MEP response, with increases in intensity above motor threshold. Furthermore, the number of repetitions at this intensity was sufficient to result in increased excitability. Change in MEP amplitude is not readily observable in most protocols since the stimulus intensity is sub-threshold. Increased MEP amplitude is a normal phenomenon in the early phase of <1Hz excitatory protocols, or a result of the relatively high-intensity stimulation used in the current protocol.

Peripheral limb movement forms a large basis for motor rehabilitation, and repetitive active movement can lead to a temporary reduction in spasticity and orthopedic benefit [29-31]. In fact, cyclic active movement can strongly increase CM excitability during muscle lengthening [3, 17, 32]. This phenomenon may be dependent on the frequency of stimulation [15] and movement rate [33]. The mechanism of action is incompletely understood, however, it is thought to result from effects of muscle spindle

afference at spinal and cortical level [4]. The strong effects of movement-related afference are transient, which suggests that active movement alone for such short duration (30 minutes) may not result in any sustained change in excitability, which would be consistent with previous results [4]. The implication for the findings of the present study is that movement during the muscle lengthening phase of movement might have therapeutic application in disorders of muscle tone and spasticity. However, more broadly, these findings suggest that the ability of PAS protocols to modulate cortical excitability may be influenced by interventions (such as movement) aimed at controlling cortical excitability.

The circumstances under which cortical and/or spinal excitability changes occur are influenced by the nature of the neuromodulatory protocol, where paired associative stimulation for example can change cortical but not spinal excitability [7]. In the present study, both spinal and supraspinal excitability changes could contribute to our findings, however, this remains to be determined. Our findings raise the question of whether this type of associative paradigm could be used to increase cortical excitability. In principle, the association of the facilitatory phase of movement with PAS repeatedly, may increase cortical excitability over time consistent with long-term potentiation, as currently is well demonstrated with PAS [7,36] yet this remains to be proven experimentally.

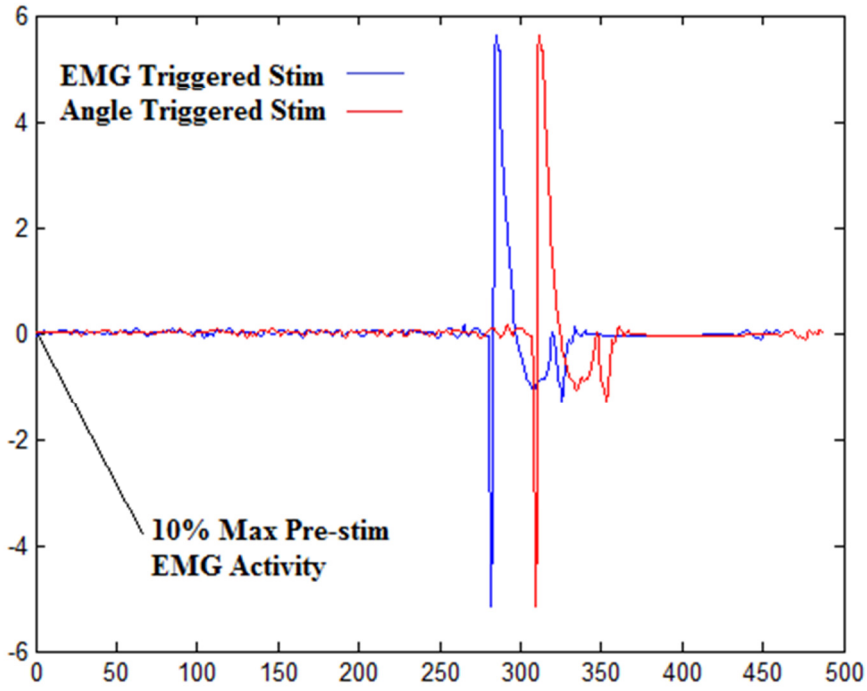

Figure 3.6 Timing of peripheral stimulation onset relative to EMG activity in target muscle. Time was calculated as the time between the target extensor digitorum muscle reaching 10% of MVC and delivery of electrical stimulation. Stimulation came ~ 30 ms *earlier* when triggered by EMG. EMG Time: 393 ± 6.69 ms; Angle Time: 419 ± 17.1 ms; Paired t-test: $p < 0.01$).

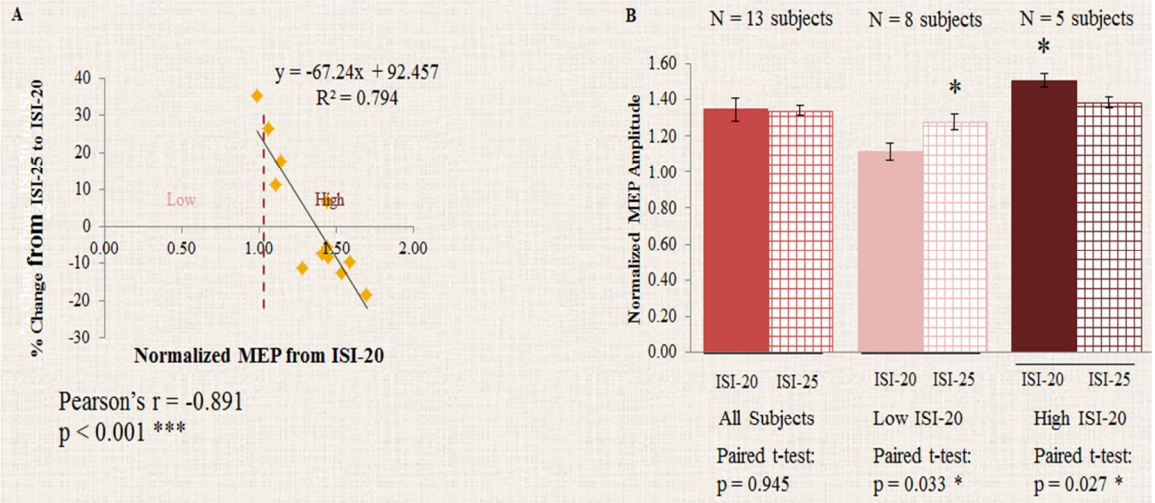

Figure 3.7 Longer ISI timing increases treatment effect only within subjects with lower baseline responses. In a set of healthy subjects, the treatment responses at ISI timings of 20 ms and 25 ms, ISI-20 (baseline) and ISI-25, respectively, were compared. The post-treatment MEP amplitude normalized to the pre-treatment MEP amplitude determined the treatment response. (A) Within this set of subjects, comparison of the responses from ISI-20 and ISI-25 by paired t-test showed no significant difference. (B) However, the percentage change from the ISI-20 to the ISI-25 response was significantly correlated with the baseline ISI-20 response. The set of subjects was then parsed into a "Low ISI-20" and "High ISI-20" groups, based on the baseline IS1-20 value being lower or greater than the x-intercept (ISI-20 = 1.38) of the linear regression (shown upper right). (C) In contrast to considering the entire set together, "Low ISI-20" individuals showed a significant increase in treatment response at the longer timing, and "High ISI-20" individuals showed a significant decrease in treatment effect at the longer timing. Based on these results, longer ISI timings only improve treatment responses in individuals with low baseline responses, and, furthermore, worsen the treatment effect within individuals with high baseline responses. Data are shown as mean ± SEM, from N = 12 subjects. * $p < 0.05$, *** $p < 0.001$.

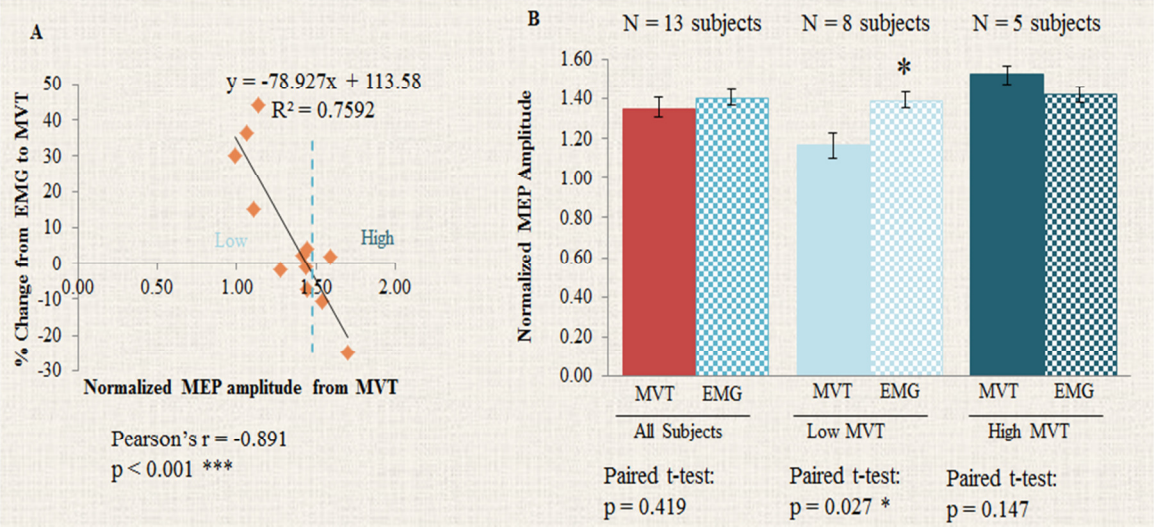

Figure 3.8 EMG-triggering of PAS stimulation increases the treatment effect only within subjects with low baseline movement-triggered responses. In a set of healthy subjects, the treatment responses when PAS was triggered by either movement (MVT) or EMG were compared. The post-treatment MEP amplitude normalized to the pre-treatment MEP amplitude determined the treatment response. (A) Within this set of subjects, comparison of the responses from MVT and MVT by paired t-test showed no significant difference. (B) However, the percentage change from the MVT to the EMG response was significantly correlated with the baseline MVT-triggered response. The set of subjects was then parsed into a "Low MVT" and "High MVT" groups, based on the baseline IS1-20 value being lower or greater than the x-intercept (MVT = 1.44) of the linear regression (shown upper right). (C) In contrast to considering the entire set together, "Low MVT" individuals showed a significant increase in treatment response using EMG-triggering, and "High MVT" individuals showed no significant change in treatment effect using EMG-triggering. Based on these results, EMG-triggering only improves treatment responses in individuals with low baseline MVT responses, with no effect on individuals with high baseline MVT responses. Data are shown as mean ± SEM, from N = 12 subjects. *$p < 0.05$, ***$p < 0.001$.

CHAPTER 4

PILOT STUDY: PAIRED ASSOCIATIVE STUMILATION IN STROKE

4.1 Abstract

Paired associative stimulation (PAS) combines electrical stimulation and transcranial magnetic stimulation (TMS). This method has been proposed to facilitate long-term changes in excitability of the cerebral cortex and potentially optimize motor recovery in stroke patients. This pilot study examined whether short-lasting changes in cortical excitability could be induced by a single session of PAS within a chronic stroke population. Two hemiparetic patients with a 100 + month history of cortical stroke were included. A 30 minute PAS protocol was applied using the parameters established Chapters 1 and 2 of this dissertation. The interstimulus interval tested was 25 ms (PAS_{25}) and the stimulation was driven by EMG activity. Both subjects completed two protocols to assess the effect of VR based PAS training on PAS-LTP like facilitation. The clinical recovery of hand function was assessed in parallel to the PAS study by the Fugl-Meyer motor scale, Wolf-Motor function test and dynamometry of finger flexion and extension. The PAS_{25} protocol induced a significant extensor digitorum motor evoked potential facilitation (25% and 49%, respectively) in both subjects on the paretic side after 30 minutes of training. Following the training, resting motor threshold (RMT) for the extensor digitorum was lowered in both subjects. The facilitation was still present 25 minutes after the conclusion of training and was accompanied by changes in clinical measurements of hand movements. These physiological and clinical findings suggest that patients with cortical infarcts may respond to PAS even several years after stroke. If the

clinical efficacy of interventions such as PAS is confirmed, it could be proposed as add-on therapy to optimize training-induced plasticity processes.

4.2 Introduction

Transcranial magnetic stimulation (TMS) has been used after stroke to investigate the integrity of the corticospinal system, the changes in the excitability of intracortical circuits, and as a potential therapeutic tool to promote recovery after stroke and improve response to standard treatments. Functional neuroimaging studies have shown that reorganization after stroke is a dynamic process [1, 2]. Transcranial direct current stimulation (tDCS), repetitive TMS (rTMS), and paired associative stimulation (PAS) which combines peripheral electrical stimulation and TMS have been shown to produce long term changes in excitability of the cerebral cortex to optimize motor recovery in stroke patients [1, 3-8]. Most studies were performed at a chronic stage in single-session studies and produced 10 to 20% functional improvement in small numbers of patients [9]. Another study used 4 weeks of daily repeated PAS in nine chronic stroke patients reported an increase of MEP amplitude and improvement in gait in some patients, but the degree of change varied widely between patients [1].

Previous results in this dissertation and other studies demonstrate that the effects of visual feedback on the motor system also contribute to the augmentation of corticomotor excitability, facilitating training and coding motor memories in healthy subjects and those with neurological pathologies [41]. These studies further show that observation of one's own movement during the training task is required to properly guide behavior and accelerate adaptations and motor learning [41,136]. This effect has also held

for instances where the subject observes movement by human-like objects that not only appear life-like, but move in a manner in accordance with normal human motion [136]. These findings have spurred advancement and development of technologies that allow researchers to utilize visual feedback, time-locked to a subject's own movements, as a means of delivering therapy to patients.

Our preliminary research shows that PAS with an ISI of both 20 and 25 milliseconds is able to facilitate lasting changes in the excitability of corticospinal projections to finger extensor muscles in 21 healthy subjects [manuscript in publication]. In this study, we examined the finger extensor muscles (ED) because the motor recovery of extension and particularly finger extension in post-stroke patients is a significant rehabilitation challenge [13]. PAS protocols focused on improving wrist extensor muscle force show reduced post stroke upper-limb disability [14, 17]. Here, we have used an extensor muscle PAS protocol in two stroke patients to investigate if changes in cortical excitability similar to what we observed in healthy subjects could be facilitated by a single session of PAS at the chronic stage of stroke. Neither the best timing for a post stroke intervention nor the ideal inter-stimulus interval for facilitation in the ED of patients impaired by chronic stroke has been determined. We hypothesized that a longer ISI would allow for consistent facilitatory results in those suffering from chronic stroke.

Virtual reality (VR) environments continue to assert themselves as a valuable component in neurorehabilitation methods. VR provides a sense of realness to subjects that can approximate the real world while allowing the researcher to vary visual parameters as well as modify size, shape, color and movements of objects in the virtual environment. VR allows for a life-like, interactive setting that is advantageous to training.

Numerous prior studies demonstrate the efficacy of VR therapy in stroke rehabilitation [35-38], and VR behavioral effects have been shown to generalize across similar, but unpracticed motor tasks [39]. VR is an ideal instrument for providing feedback in neurorehabilitation protocols.

For these reasons, this offers an ideal starting point for a systematic investigation of the effects of PAS on corticomotor excitability in stroke recovery. We hypothesized that applying a virtual reality (VR) based single pulse EMG driven PAS protocol combined with voluntary movement and an ISI of 25 ms would lead to LTP-like plasticity effects similar to that observed in our healthy subjects. We also predicted that by increasing the ISI from 20 to 25 ms, we would observe a corresponding augmentation of the increases MEP amplitude due to the longer stimulation latencies observed in those affected by stroke. Our long term goal is to use this knowledge to develop a robust, novel VR based PAS platform as a tool for neurorehabilitation.

4.3 Methods

4.3.1 Subjects

Two subjects were studied who had chronic, stable hemiparesis. At the time of testing, one of the subjects had been undertaking physical therapy that was stopped prior to and for the duration of the study. Subject details are summarized in Table 4.2. Subjects were assessed with hand function tests on two occasions 1 week apart before the intervention and 1 time after the training. Paired associative stimulation training lasted for 30 minutes. Electromyography measurements were made prior to the intervention and all

measurements were repeated at the end of the training. Follow-up measurements were repeated after completing the protocol.

Table 4.1 Stroke Subject Demographics

Subject	Age	Impaired Hand	Lesion Location	Handedness	Months Post -Stroke
1	63	L	R cortical	R	179
2	71	L	R cortical	L	93
3	57	R	L cortical	R	62
4	69	R	L cortical, L subcortical	R	168

4.3.2 Paired Stimulation Protocol

The paired stimulation paradigm combined TMS and peripheral electrical stimulation of the extensor digitorum. The peripheral stimulation was a single 1-millisecond shock triggered by EMG activity of the ED described in Chapter 3 which repeated every 4-6 seconds over the belly of the extensor digitorum through surface electrodes. TMS was delayed by 20 or 25 milliseconds (interstimulus interval, ISI) with respect to the onset of the electrical pulse. Both TMS and electrical stimulation were applied at an intensity that evoked a just-visible motor response in the extensor digitorum.

Hand position was relayed by a Cyberglove (CyberGlove Systems) wired 22 sensor data glove. All hand movements were tracked by the glove which was calibrated for each subject (VirtualHand Software, CyberGlove Systems). Once calibrated, the

subject's hand movements were shown in real time on the display. Each subject was asked to follow the onscreen OPEN/CLOSE instructions to open and close their hand fully at their normal rate. On screen targets were displayed to prevent hyperextension and hyperflexion of the fingers. EMG activity during maximum voluntary contraction (MVC) of the ED was measured prior to the experiment. A custom built MATLAB program recorded EMG activity during the training and paired stimulation was triggered by EMG activity equal to between 15-30% of maximum voluntary contraction in the ED.

4.3.3 Electromyography

Surface electromyographic (EMG) activity was recorded from five wireless Trigno™ electrodes (Delsys Inc.) placed over the muscle belly of the right extensor digitorum, right flexor digitorum, right flexor digitorum indicis, right extensor indicis and the abductor digit minimi. EMG signals were amplified and band-pass filtered before being digitized at 1000 Hz. EMG was recorded continuously and then processed with each MEP comprising a window spanning 50 ms prior and 100 ms after electrical stimulation using a custom built MATLAB acquisition and analysis system (Mathworks Inc.).

4.3.4 Neuronavigated Magnetic Stimulation

Single-pulse TMS (Magstim Rapid2, 70mm double AFC coil) was applied at 100% of the resting motor threshold, the minimum intensity required to elicit MEPs > 50 μV in the right extensor digitorum (ED) muscle in 4/6 consecutive trials. For subjects who had undergone MRI, a high-resolution anatomical MRI scan (3T Siemens Allegra) was used to render a 3-D cortical surface. A visor with motion tracing markers was placed on the

subjects head. Fiducial locations on the MRI were core-registered with the subjects head to allow frameless neuronavigation (Visor, Advanced Neuro Technology). The optimal site of stimulation for ED (i.e., the hotspot), determined from initial exploration, was defined as the site with the largest MEPs for a given supra-threshold stimulus intensity, and used throughout the experiment. The stimulated ED hotspot of the motor cortex was marked on the MRI scan. The coil was held tangentially with the handle facing 45º posteriorly off the sagittal plane, and was tracked online to be stay over the ED hotspot. For subjects without an anatomical MRI, their head was co-registered to a model MRI.

Two hundred and fifty TMS pulses were delivered to the ED hotspot at 110% RMT with a frequency of approximately 0.14 Hz. The level of attention, a significant modulator of PAS-LTP effects [16], was controlled and attention was maximized to the simulated hand by a color change on the virtual display when the command changed. Subjects were asked to count and report the total number of stimuli they received as correctly as they could at the end of PAS.

In the two subjects, the interventional paired stimulation was performed with electrical stimulation of the radial nerve by placing the bipolar electrodes just proximal to the muscle belly of the ED. Intensity was chosen as 110% of that sufficient to produce a just noticeable twitch in the ED muscle at rest (9.5 ± 0.5 mA, 300 V_{max}, n =2). Stimulus was performed with a Digitimer DS7A stimulator (Digitimer Ltd.) using constant current square wave pulses (cathode proximal, stimulation width 1 ms) followed 25 ms later by TMS. Pulse PAS consisted of 250 stimulus pairs at a frequency of 0.14 Hz.

4.3.5 Neurophysiologic Tests

Cortical excitability of the primary motor ED area was assessed with single pulse TMS before (pre) and after (post) associative stimulation. The MEP amplitude (20 x 2, 0.16 Hz) was measured before and at 0 and 30 minutes after PAS for both subjects. To clarify the presentation of some results, the size of MEPs after PAS was normalized to MEP pre-value and expressed as mean percentage.

Resting motor threshold (RMT) was measured before and after PAS. RMT was defined as the minimum TMS intensity (measured to the nearest 1% of the maximum output of the magnetic stimulator) required to elicit a MEP of at least 50 μV in the relaxed ED in at least 5 of 10 trials with an inter-trial interval of 7 seconds. RMT measurements after PAS were performed between the two post MEP amplitude measurements, during the 30 minute delay after the end of the intervention and not at the end of the experiment. For each session and both patients, the PAS protocol was always applied in the morning, between 9:00 am and 12:00 pm to avoid any diurnal variations of PAS effect [18].

4.3.6 Virtual Reality Simulation

The VR simulation was built using Virtools 4.0 software package (Dassault Systems) and communicates with the data glove through the open source VRPN (VR Peripheral Network) interface. The virtual hand models in the VR simulation closely matched the subject's hand size. The display showing the virtual scenario includes a simple "Open" or "Close" instruction above the hand models, which cues subjects to perform the task or rest. Immediately before the experiment, subjects were trained to move their hand with

veridical VR feedback to get familiar with the mapping between their motion and the VR hand's motion. Subjects were instructed to begin each trial with their fingers in a relaxed, flexed and adducted, position with each finger joint angle at approximately 90º flexion. Hand position was recorded by a Cyberglove (CyberGlove Systems) wired 22 sensor data glove. All hand movements were tracked by the glove which was calibrated for each subject (VirtualHand Software, CyberGlove Systems). Once calibrated, the subject's hand movements were shown in real time on the display. Each subject was asked to follow the onscreen OPEN/CLOSE instructions to open and close their hand fully at their normal rate. Subjects were given 3 seconds to perform the extension movement and 3 seconds to return to the rest position. Subjects were asked to attend to the visual feedback while maintaining consistent movement behavior across all trials. To reduce the likelihood that subjects would alter their motion across the different trials we provided a visual target toward which the subjects produced their movement.

The subject was seated with their right arm supported at the hand, wrist and elbow in a custom built armrest. The wrist was slightly flexed and the apparatus allowed for 90º of finger extension/flexion. The forearm was positioned partially supine on a curved, padded rest so that the ulnar styloid sat just on the edge of the padding. Their arm was positioned such that the subject's arms were aligned with a pair of onscreen virtual hands created using Virtools Software (Dassault Systems) on a TV display. The fingers were placed in a passive, flexed position at the MCP, PIP and DIP joints. To maximize the perception of realness, the TV was positioned horizontally above the hands and angled so that the vantage point of the virtual hands, which was driven by glove data, corresponded to the subject's actual hands underneath the TV. Our published and preliminary data

suggest that our VR environments provoke a sense of reality to the subjects, provide valid and reliable measures of hand kinematics, and possess potential as a rehabilitation tool in clinical populations [35, 36].

To evaluate and test for functional changes during the study, each subject was assessed with the Fugl-Meyer motor scale of the upper limb, the Wolf Motor Function test and dynomometry of finger extension using a Psytech Finger Flexion/Extension Gauge. Assessment was made 1 week prior to the first PAS session, the day of the first PAS session, and 1 hour after the PAS session.

4.3.7 Statistics

To determine the effect of each PAS protocol on MEP amplitude MEP pre, MEP post and MEP Electrophysiological variables (MEP, EMG) were averaged across trials for each condition and subject. Means were submitted to a repeated-measures analysis of variance (rmANOVA). Two paired t-tests were used to compare pre and post PAS MEP amplitudes across both protocols (using a Bonferonni correction). Finally rmANOVA was also performed for to characterize the degree to which interstimulus interval (ISI) contributed to the MEP excitatory effect. For this, MEP was defined as the dependent variable ISI as the independent variable. Data were analyzed with PASW Statistics 18 (SPSS). rmANOVA was used to test for main effects and interactions. Statistically significant interaction effects were tested post hoc by Tukey's honestly significant difference (HSD) test. Significance threshold was set at $P < 0.05$. Changes in clinical score (FMS) and in finger extension force (dynamometer) between the three sessions (SESSION effect: M1, M5, and M12), a nonparametric Friedman test was used and post

hoc analysis was performed with the Wilcoxon test. At each session changes of the RMT before and after intervention was analyzed by a Wilcoxon test. Correlation between changes of MEP size after PAS (normalized to the MEP pre-value) and changes of RMT, the FMS, and the wrist extension force data were also investigated using a nonparametric Spearman test. For all tests, the level of significance was set at $P = .05$.

4.4 Results

Thresholds and latencies for both weak and normal muscles did not significantly change throughout the testing period. On average, there were increases in both MEP amplitude and the level of EMG activity recorded during MVC in the muscles in the affected leg (Table 4.2). However, the degree of change was extremely variable between subjects and the increase in MEP amplitude and EMG levels did not reach significance in the grouped data. Given this variability, we analyzed all of the variables within each individual across the course of the study. Analysis of individual data revealed that, for the affected limb, MEP and MVC amplitudes were consistently elevated in five of the nine subjects ($p < 0.05$).

The results showed that pulse stimulation protocols resulted in greater increases in excitability as measured by MEP amplitude when compared to rest protocols. A 2x2 repeated measures ANOVA was performed to determine if stimulus type (pulse vs. train) during PAS intervention had an impact on MEP amplitude. The results show that stimulus does have a significant ($p < 0.05$) effect on PAS-LTP like effects. The data suggest that pulse protocols should be used for interventions with stroke subjects to maximize rehabilitation potential related to plasticity and excitability changes.

Table 4.2 Resting Motor Threshold Changes

Subject	Stimulator Output Movement-Pulse EMG 20	Stimulator Output Movement-Pulse EMG 25	Time-point
2	68	66	PRE
2	64	64	POST
1	85	81	PRE
1	79	77	POST

In this study, analysis of the additional muscles (FD, FDI, EI ADM) recorded showed no significant changes in MEPs obtained (Figure 4.4). It has frequently been proposed that PAS-facilitated adaptation represents a form of plastic neuromodification that is synapse- specific [180-182]. The idea of topographical specificity [91]suggests that changes in excitability brought about by PAS protocols are largely restricted to the cortical representations of muscles innervated by the peripheral nerve that was stimulated electrically [88, 156].

The results of this study support the concept of topographical specificity. The only non-target muscle to exhibit measurable MEPs was the extensor indices, which is innervated by the posterior interosseous nerve, a distal branch of the radial nerve that supplies the ED. However, while the trend in MEP amplitude appeared to be an increase as in the ED, the data was statistically unreliable to make any definitive conclusions.

Table 4.3 Subject Clinical Profiles

Subject	FMS (Upper Limb)	FMS (Hand)	WMFT time (sec)	Finger Flexion (kg F)	Finger Extension (kg F)	MEPs Elicited
1	30/66	10/14	75.3	16±2.0	2.0±0.05	Yes
2	53/66	13/14	33.71	38±1.0	3.1±0.20	Yes
3	35/66	8/14	119.02	31±1.5	0.6±0.14	No
4	31/66	8/14	93.08	39±3.7	0.7±0.28	No

Abbreviations: FMS, Fugl-Meyer motor score; WMFT, Wolf Motor Function test; MEP, motor-evoked potential

4.5 Discussion

This pilot study is the first report of the use of a dual peripheral and central stimulation paradigm to induce functionally beneficial changes in the excitability of the finger extensors in stroke patients. However, the effects of the intervention were not significant in the functional or neurophysiologic indexes, which is probably the result of the small sample size, the heterogeneity of subjects' initial clinical scores and the inherent variance in motor evoked potentials It is unlikely that the improvements in neurophysiologic and functional measures reported here are due to neuronal regeneration, given the time scale of change. A more likely explanation for the changes in corticospinal excitability is the unmasking of previously silent corticocortical or corticosubcortical connections. The mechanisms by which this is brought about may include both a reduction of local inhibition and changes in synaptic efficacy. Periods of prolonged peripheral nerve

stimulation produce changes similar to those seen with the paradigm described here and it has been proposed that the mechanisms behind these changes may depend on alterations in the efficacy of GABAergic synapses [113, 129].

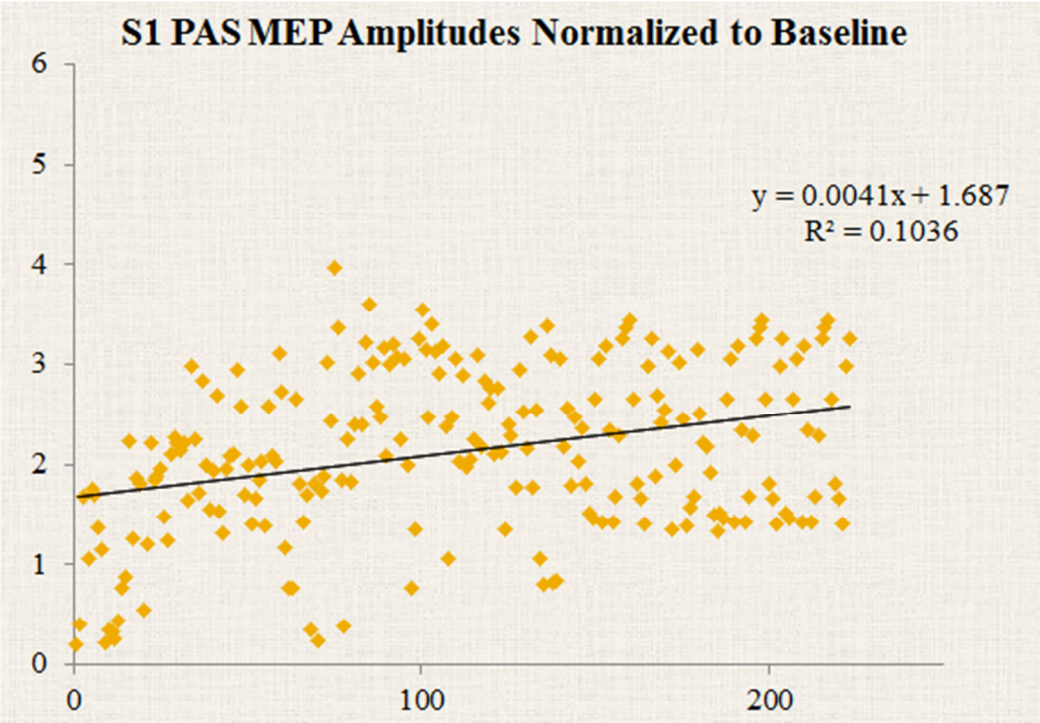

Figure 4.1 Motor-evoked potential amplitude recorded during stimulation.

The nature of the changes induced by the dual stimulation paradigm (i.e., persistent but reversible and topographically specific) also suggests a role for long-term potentiation [12]. The changes in function could be the result of a nonspecific placebo effect, but there are a number of reasons why this is unlikely. First, subjects were included only if they had not been receiving regular physiotherapy and were considered to be functionally stable for at least 6 months and, in both cases of those who participated in the intervention, had been stable for several years. The similarity between the two baseline functional scores further demonstrates that these subjects were functionally

stable. Second, many of the parameters that improved in both subjects, such as maximal MEP amplitude and resting motor threshold, are objective and unlikely to be subject to modulation as the result of a placebo effect. Third, functional measures improved more in the subject in whom neurophysiologic measure improvements were larger. These functional measurements are not reliable given the duration of the intervention (2 protocols spread over 2-4 weeks, 60 minutes total stimulation) so we are unable to report any functional improvements.

Table 4.4 Wolf Motor Function Item Times (Pre session and Post session)

Subject	S1			S2		
	Pre	**Post**	**%**	**Pre**	**Post**	**%**
Forearm to table side	0.97	0.99	-2.0618557	0.98	0.83	15.30624
Forearm to box	1.31	1.01	22.9007634	1.03	0.91	11.65044
Extend elbow side	0.5	0.64	-28	0.68	0.7	-2.941177
Extend elbow side weight	0.65	0.47	27.6923077	0.58	0.42	27.58629
Hand to table front	0.34	0.34	0	0.56	0.57	-1.785719
Hand to box front	0.39	0.86	-120.51282	0.87	0.29	66.6667
Reach and retrieve	3.19	2.37	25.7053292	5.13	2.35	54.19103
Lift can	4.44	3.67	17.3423423	5.27	4.07	22.77039
Lift pencil	3.53	2.56	27.4787535	2.16	2.96	-37.0370
Lift paper clip	5.5	2.98	45.8181818	2.57	1.9	26.07009
Stack checkers	9	120	-1233.3333	86.86	45	48.19247
Flip cards	18.17	23.64	-30.104568	10.97	8.08	26.34461
Turn key	6.09	6.7	-10.01642	3.41	3.69	-8.21137
Fold towel	120	17.34	85.55	21.28	13.16	38.15747
Lift basket	5.53	5.38	20.79566	4.08	3.03	25.73521
Sum times	179.61	187.95	-4.643394	146.43	87.96	39.93031

Many factors may have contributed to the highly variable response pattern to the intervention. The age, size and site of lesion, and the time since stroke in the subject population varied considerably. These factors may be critical in determining the nature of

the response to the given intervention [8]. However, with this small sample size; it is not possible to determine factors that could be predictive of treatment outcome. Attention is also known to have a major influence on motor learning and cortical reorganization [10]. Whereas the subjects selected were judged to have no significant cognitive deficit, it is possible that some paid more attention to the stimulus. It is also possible that subjects with more positive outlooks were prepared to try harder to achieve their optimal performance during testing. Even though only a limited number of functional scores showed improvements across the group, most of the subjects showed an increasing trend for their scores, which may indicate clinical significance. Thus, we suggest that these results are sufficiently encouraging to extend this study to a larger stroke population with a view to determining what characteristics are associated with positive outcomes.

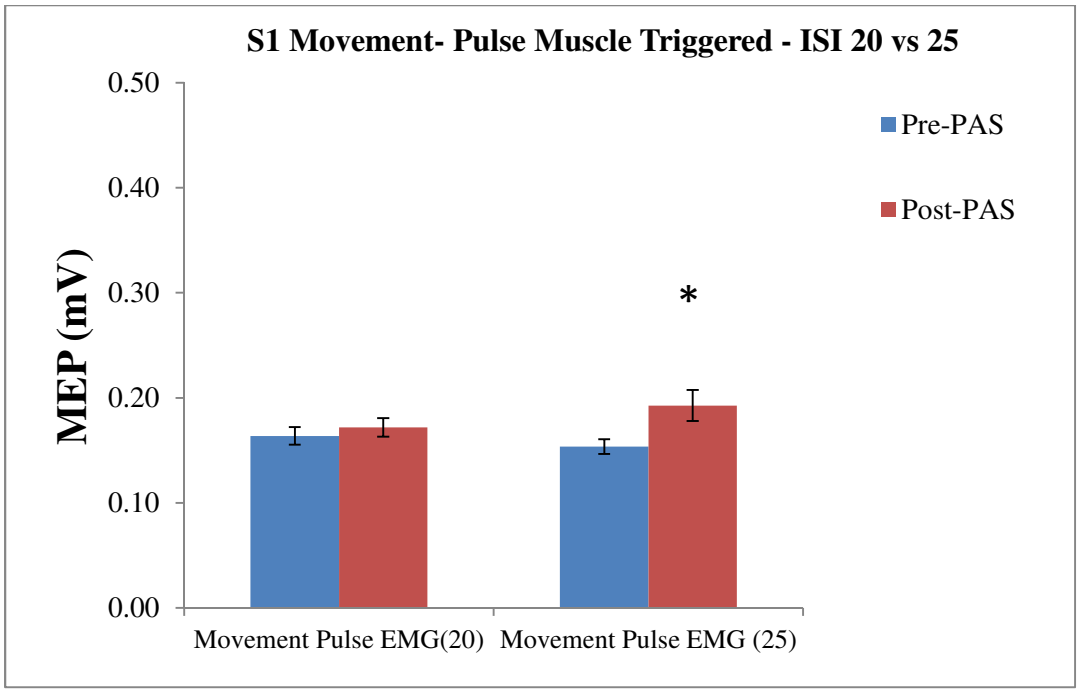

Figure 4.2 Stroke subject 1: pre vs. post MEP amplitudes. * indicates t test significance for changes ($p < 0.05$) pre to post.

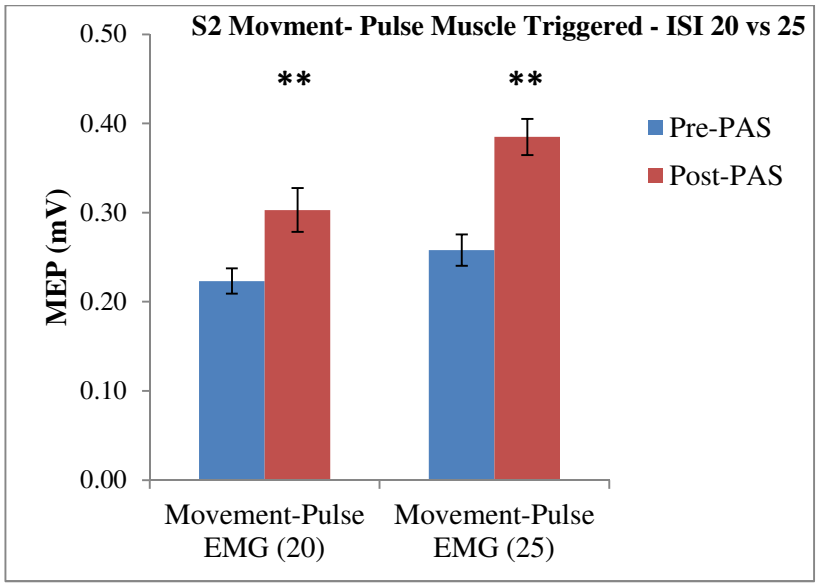

Figure 4.3 Stroke subject 2: pre vs. post MEP amplitudes. ** indicates t test significance for changes ($p < 0.01$) pre to post.

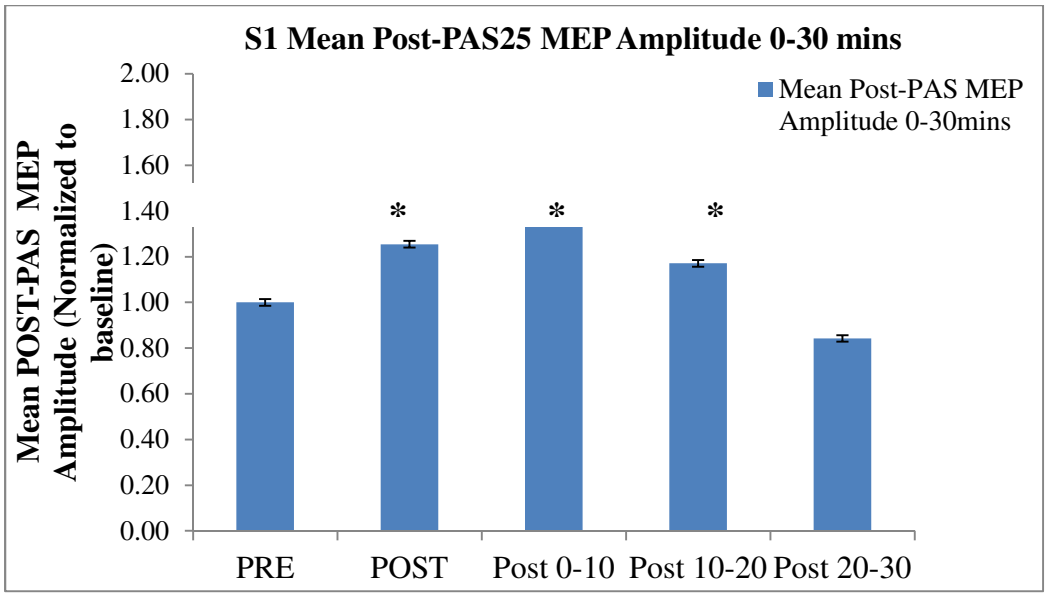

Figure 4.4 Excitability retention in stroke subject 1. Effects are seen to outlast the duration of the stimulation protocol and persist for up to 20 minutes. *denotes significant elevation from pre MEP amplitude levels ($P < 0.05$).

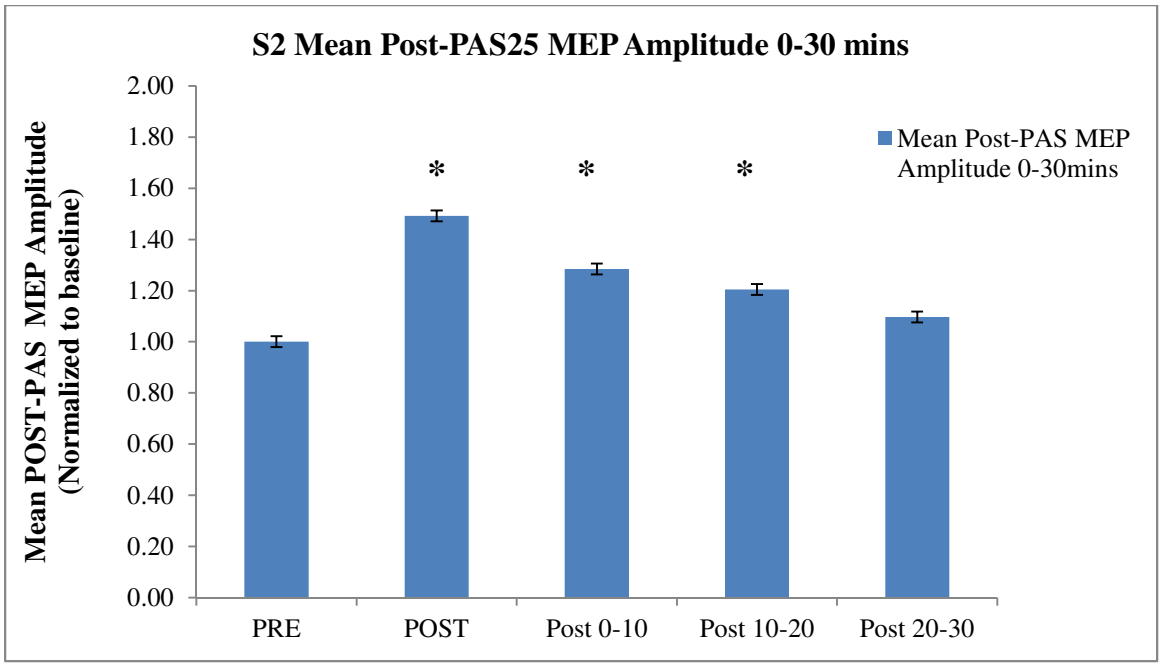

Figure 4.5 Excitability retention in stroke subject 2. Effects are seen to outlast the duration of the stimulation protocol and persist for up to 20 minutes. * denotes significant elevation from pre MEP amplitude levels ($P < 0.05$).

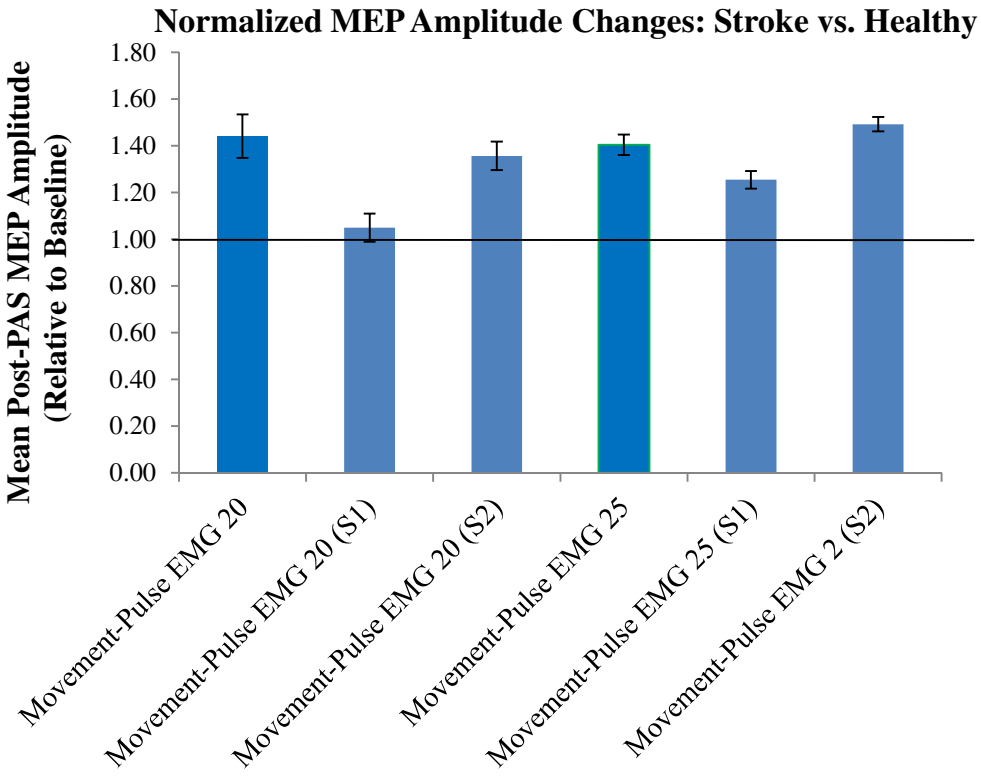

Figure 4.6 Group excitability changes for all tested protocols. The line indicates no change in motor-evoked potential amplitude post-stimulation.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSION AND SUMMARY

5.1 Discussion

Research has made significant progress in the field of non-invasive brain stimulation, starting from the observation that both facilitatory and inhibitory PAS effects may persist after the induction of plasticity. Compared to other stimulation paradigms such as TBS and rTMS, PAS seems to be the most efficient protocol [58] and, a logical extension of this will be attempts to use PAS as a therapeutic tool in neurologic and psychiatric disorders characterized by dysfunction of distinct brain networks such as Parkinson disease. In this dissertation, we showed that the influence of several major factors such as intracortical facilitatory and inhibitory networks as well as the parameters of stimulation (number of pairs) on the effect of PAS. However, there are other variables that may affect the PAS response. Attention [252], cortisol level [230], circadian cycle [229], dopamine level [260]and age [81] may influence the PAS effect as well as various PAS parameters such as intensity of median nerve stimulation, repetition rates and ISIs, just to name a few. The finding that PAS response is exaggerated or diminished in certain diseases and that the certain medications for example dopaminergic drugs can modulate the PAS effect all indicate the possibility of clinical application of this technique as a noninvasive predictor of the clinical response after treatment or as a diagnostic tool.

Although we answered few of these questions, further studies are required to investigate the complex interactions between brain, PAS and other environmental factors in both healthy and diseases.

5.1.1 Effect of Motor Practice on MEP

In general MEP amplitude recorded from muscle groups involved in training movement increases [35, 176]. Increases in MEP amplitudes are often associated with improved performance or changes in the kinematics of movements elicited by TMS of M1 after training protocols [35, 176] and may reflect changes in the motor output zone related to motor learning. Muellbacher et al. [176] studied the learning-related changes in M1 excitability with TMS while and found that subjects rapidly learned to optimize ballistic contractions measured via pinch acceleration and peak force and improvement in subjects' performance were associated with concomitant increase in MEP amplitudes in targeted muscles. MEPs returned to their baseline amplitude after subjects had acquired the new skill, no practice induced changes in MEP amplitude were observed with task over learning [176]. These findings are consistent with concepts of multiple overlapping motor representations in animal studies of motor cortex [70, 233]. Intracortical microstimulation of macaque monkey motor cortex showed extensive, horizontally oriented, intrinsic axon collaterals that provide inputs to many different forelimb movement representations these neurons may be recruited during complex movements to coordinate the activity of motor cortical zones during a use dependent plastic change in motor cortex [120].

Use-dependent and skill-dependent plasticity contributes to the recovery of motor function after injury to the brain [184, 185] and this functional plasticity of the motor cortex accompanied by changes in synaptic morphology in animal models [185]. These findings set the stage for development of new, more effective rehabilitation interventions. Cortical stimulation can enhance the beneficial effects of motor training on performance,

cortical plasticity and motor cortical excitability [119, 134]. In contrast to the previously described beneficial effect cortical stimulation in recovery of stroke related loss of use-dependent plasticity [119, 134] we found that in healthy subject cortical stimulation did not further improve increased MEP amplitudes after use-dependent plasticity and even resulted in homeostatic reduction of MEP amplitudes after increasing the amount cortical stimulation. Cortical stimulation did not affect motor learning task performance either. Possible explanations for this paradoxical findings could be 1) cortical stimulation may improve loss of function in a pathological condition but not necessarily improve MEP amplitudes or motor behavior performance in already optimally functioning healthy subjects; 2) it can also possible that improvement in behavioral effect occur in different time scale (for example weeks or months after cortical stimulation and motor practice) previous studies had shown correlation between MEP amplitude and improvement in kinematics of movement. In our study of healthy subjects preconditioning of use-dependent plasticity with cortical stimulation at higher number of stimulation pairs resulted in reduction in MEP amplitudes. This might indicate that healthy subjects have already reached their best performance and further increase in performance is not possible and increases the possibility of first explanation for our findings.

5.1.2 Variability in TMS response

Inter- and intra- individual variability exists in most TMS measures. Much of the TMS studies assume little difference between individuals in order to compare healthy subjects with groups of patients or the effect of a particular intervention on the MEP. Although age and sex are commonly matched between groups the rest of influencing factors are

often being neglected. Intra-individual variability is usually considered as 'noise' which is a naive assumption as critical information might lie within these changes of variability in one subject. This issue recently attracted some attention. For example, one study showed that iTBS increased performance variability, which correlated with learning outcome and suggests that increased motor output variability may have role in improvement of performance after iTBS [257].

Age is another important factor for inter-individual variability. Response to cortical stimulation interventions can be affected significantly by age. One study showed that the magnitude of MEP increased by PAS in the young and middle but not in the elderly and its change was negatively correlated with the age. These results suggest that the human M1 shows age dependent reduction of cortical plasticity [81]. Decreased M1 excitability maybe caused by reduced intracortical circuits responsiveness or disruption of sensorimotor integration or both. Attenuation of in paired pulse intracortical inhibition or changes with age has not been confirmed yet [277]. In this dissertation looking at the PAS responses in groups of subjects in Chapters 3 and 4 indicate significant variability between subjects. Part of this difference can be explained by the difference between average age of subjects participated in different experiments. Genetic factors also participate in significant inter-individual variation of responses of the brain to TMS. One study showed that individuals with the val66met polymorphism in the brain-derived neurotrophic factor (BDNF) gene show less increase in the MEP after motor training [164]. Other factors that can participate in the inter-individual variability of brain to TMS are gross anatomy of human scalp, and distance between motor cortex and surface of the head [254, 162].

5.1.3 Paired Associative Stimulation

In our experiment similar to previous studies we found an increase in the size of the MEP amplitude, as well as an increase in the duration of the CSP recorded from the contracting target muscle [200, 251, 252, 74]. Therefore, PAS-induced plasticity, although may influence active neuronal circuits involved in GABAB receptor mediated inhibition. In one study [143] using current direction to preferentially activate early or late I waves after PAS authors found that the increased effectiveness with use of anterior to posterior current direction in PAS over posterior to anterior current direction which suggests I3 input to corticospinal neurons which selectively more active with anterior to posterior current direction has an important role in induction of associative plasticity in the human motor cortex. In this way, PAS-induced plasticity may be different from TBS-induced plasticity which appears to rely on modulation of the early I-waves [116]. Our results add to these findings as we demonstrate selective reduction of PAS effect by engaging in interstimulus intervals below 20 ms and using trains of stimulation.

5.1.4 Safety

Safety and tolerability are key issues not just for the risk-benefit ratio assessment of novel therapeutics, but also for their impact on patient commitment and compliance with a time consuming brain stimulation paradigm. In order to increase subjects compliance, we used 0.2 Hz frequency for PAS paradigms used in this dissertation instead of 0.1 Hz used in original PAS study by Stefan et al. [251] It is also important to investigate the effect of single vs. multiple sessions of brain stimulation to understand the magnitude of additional sessions of stimulations on the measure of interest. We strictly followed published safety

guidelines [220] for TMS. TMS in general is a very safe and thousands of people have had the experience with no adverse effects although seizures have been reported in few cases. The common side effect is usually limited to local pain as a result of the pressure of the coil, mild headache and possibly transient hearing changes as result of discharge related noise. In this dissertation we found no major or minor adverse effects of PAS which increase the favorability of this technique for potential clinical applications.

TMS is a great tool because of its safety record, temporal resolution and because it makes it possible to manipulate brain activity in human non-invasively. However, certain limitations exist for the majority of TMS studies:

Poor spatial resolution both as a result of the limited focality of stimulation as well as the conventional localization of the area of interest according to 10-20 EEG system or based on the motor hotspot. Several streams of research are underway to tackle these issues by improving the focality of TMS coils and also by combining imaging (e.g., MRI) with TMS [243] to improve spatial resolution and use of optically tracked frameless stereotaxic neuronavigation systems, which incorporate individual MRI data to deliver TMS in anatomically precise locations.

Cellular mechanisms underlying the TMS induced events are not well understood. Several studies have used receptor agonist and antagonist to derive plausible mechanistic explanations for TMS induced interactions. However, the majority of these studies had significant limitations because of the small number of drugs that are available to be tested safely in human. Simultaneous observation of PAS effect at cellular level may be necessary to provide definitive evidence for the underlying mechanism of actions of this paradigm.

The application of TMS to excite a cortical process and deducing the relevance of that area in performance of tasks is also a complex issue that needs to be addressed. TMS induced impairment of task performance could be the result of different chains of effects: TMS can increase the function of an area that inhibit the task performance or disrupt the function of an area that facilitate the task performance, or to inhibit or excite an area of the brain that compete or promote with the region of the brain relevant to process under the study. These chains of event are crucial in the interpretation of the results from TMS studies [195]. Metabolic changes measured by PET and blood oxygen level changes using fMRI both showed TMS induced changes [226, 19, 255]. TMS may be used to manipulate brain function to narrow down brain-behavior relationship to functionally relevant hypotheses. Understanding advantages and disadvantages of this technique are necessary to interpret result of TMS studies and to design new ones. In this dissertation, we tried to relate our understanding of the mechanisms of neuronal plasticity at the cellular level to the system and behavior level. The next logical step would be to use findings of this study and apply them to the patient population – from bench to the bedside.

5.2 Conclusions

The association of somatosensory afferents from the actively moving limb with PAS targeted to the ED and primary motor cortex in healthy human subjects can be used to modulate corticomotor excitability, capable of outlasting the intervention period by several minutes. Several parameters of PAS stimulation (stimulation rate, intensity, duration, pulse-type) and behavior (movement vs. rest, number of repetitions) have been

identified for optimal effect. Other PAS parameters (e.g., ISI, the timing of stimulation with regards to movement) require further investigation for the development of the optimal protocol for the ED. These findings suggest that movement be determined whether these findings could be applied to the treatment of neuromotor disorders involving altered ED muscle tone such as dystonia, spasticity, muscle weakness and other sequelae of stroke, these findings inspire further research to optimize therapeutic applications of PAS in patients with neurological deficits to modify synaptic transmission more effectively than presently possible.

5.3 Limitations

Although these current studies demonstrate a correlation between several PAS parameters and corticomotor excitability in both health subject and stroke patients, a demonstrable relationship between this excitability and neuroplastic changes (cortical reorganization) and behavioral changes has not yet been determined. Additional TMS measurements and techniques (cortical organization maps) would allow for the further elucidation of the relationship between cortical excitability, neural plasticity and behavioral changes.

PAS is an excellent rehabilitative tool because of its safety record, temporal resolution and because it makes it possible to manipulate brain activity in human non-invasively. However, certain limitations exist for the majority of PAS studies: Poor spatial resolution both as a result of the limited focality of stimulation as well as the conventional localization of the area of interest based on the motor hotspot. Adding another measure of cortical-motor activation and organization such as functional magnetic resonance imaging (fMRI) or electroencephalography (EEG) would help

address these issues by improving the focality of TMS coils and also by combining imaging with PAS to improve spatial resolution.

5.4 Future Directions

In Chapter 2, we showed that behavior during intervention and stimulation type plays an important role in maintaining cortical excitability. We demonstrated the conditioning effect of voluntary movement on PAS paradigms. It would be interesting to assess the observed effects using pharmacological manipulations to block the GABAergic interneurons and assess the conditioned PAS paradigm to confirm if it is possible to reduce the effect of LTP due to PAS. In Chapter 3, we found a graded response to PAS with different ISI and also an interaction with motor learning. The EMG triggered stimulation is likely to stimulate the muscle early in the movement phase, which has been shown to improve excitatory effects of TMS protocols [68]. Our lack of kinematic hand movement data time-locked to EMG activity does not allow us to quantify this time difference. Thus, it would be interesting to conduct PAS experiments comparing EMG and movement triggered PAS with the kinematic measurements synchronized with EMG collection to determine if there is a significant difference in the delay between the two protocols.

In Chapter 4, we found a graded response to PAS after the intervention was over as well as a possible interaction with ISI. While we saw no changes in clinical the measurements or functional assessments after 30 minutes of training, given the limited sample size and short duration of the pilot study, further collection of chronic stroke date could yield statistically significant results. Our protocol was also only 30 minutes per day

and a total of four sessions across 28 days. Homeostatic interactions theoretically happen at longer time scales, it would be interesting to perform PAS protocols for a longer period of time and to follow them to look for functional changes. Improvements in motor learning can also occur on lengthier time scales [207]. Interactions with motor learning tasks in chronic stroke patients are the logical next step to take with the movement pulse PAS paradigm. The fact that we did not observe changes in hand function or test for motor learning does not exclude the possibility therefore studies with longer training schedules and longer periods of follow-up are warranted. Understanding the rules of synaptic plasticity at the systems level will ultimately help to develop effective protocols to modulate the motor cortex or new markers to capture defects of cortical plasticity in patients with neurological disorders.

Behavioral aspects of PAS were investigated Chapter 4 but hand kinematics were not adequately observed. Further experiments involving measurements of hand motions (e.g., gripping tasks, functional tasks) measuring peak finger acceleration and variability of finger strength and hand path could be of physiological importance [103] because learning processes might require increased motor variability as an inherent feature for performance improvement, planning and learning. Further studies are required to compare different protocols in their behavioral aspects. It will be of interest to investigate LTP PAS protocols as it relates to their behavioral correlates and motor learning variability.

Although tools like PAS present great therapeutic potential, the realization of that potential requires understanding of pathophysiology of illness of interest, and of the mechanisms by which brain stimulation paradigms can induce plastic changes in the

functioning of those abnormal circuits. TMS is a focal intervention and as a result its clinical utility will depend upon our knowledge of the intracortical networks in the underlying disorder. This dissertation helps with understanding of part of these mechanisms and corticomotor processes. Some of the implications of current dissertation and potential applications are as follows: GABAergic system is involved in PAS LTP-like effects and given that GABAergic signaling in the motor cortex plays an important role in the development of perilesional or use-dependent plasticity after stroke, a PAS paradigm could potentially have a significant response in this population of patients in compared to healthy controls. As mentioned previously, PAS with longer duration of stimulation may induce homeostatic responses. This is of clinical relevance because it may provide new avenues for rehabilitation medicine. Improvement after stroke and spinal cord injuries should be studied as potential targets for interventions to improve motor learning especially with longer period of observations and multiple stimulation sessions. Increased corticomotor excitability and improved RMT observed after PAS in Chapter 4 also indicates that PAS could produce clinical effects in patients after strokes by network reorganization and boosting the motor output.

An ideal method to deal with the possibility of undetected PAS induced changes as the result of limited temporal resolution of imaging techniques is to combine EEG measures with PAS to identify these effects. PAS and TMS may be used to manipulate brain function to narrow down brain-behavior relationship to clinically relevant propositions. Understanding strengths and weaknesses of this technique are necessary to interpret result of PAS studies and to design new ones. In this dissertation, we tried to relate our understanding of the mechanisms of motor excitability at the cellular to the

system and behavior level. The next logical step would be to use findings of this study and apply them extensively to the stroke population.

5.5 Summary

Understanding how PAS protocols effects the motor system could be essential for designing effective rehabilitation interventions for those neurologically impaired by stroke. This current project demonstrates the efficacy of incorporating and testing several PAS parameters and visual feedback mechanisms, like those used in our virtual reality therapy techniques. Training with PAS not only elicits increases in motor excitability, but the virtual environments allow for the easy design of many difference feedback mechanisms and training tasks that motivates patients to perform movements accurately and consistently, assisting in any potential recovery. The author shows here that PAS training with a movement single pulse design may produce sustainable excitability and resting motor thresholds that are closer to pre-stroke levels. The capacity to induce focused excitability and decreased RMT in response to this PAS training suggests that virtual reality PAS therapy may be a more efficacious form of neurorehabilitation compared to traditional task training.

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